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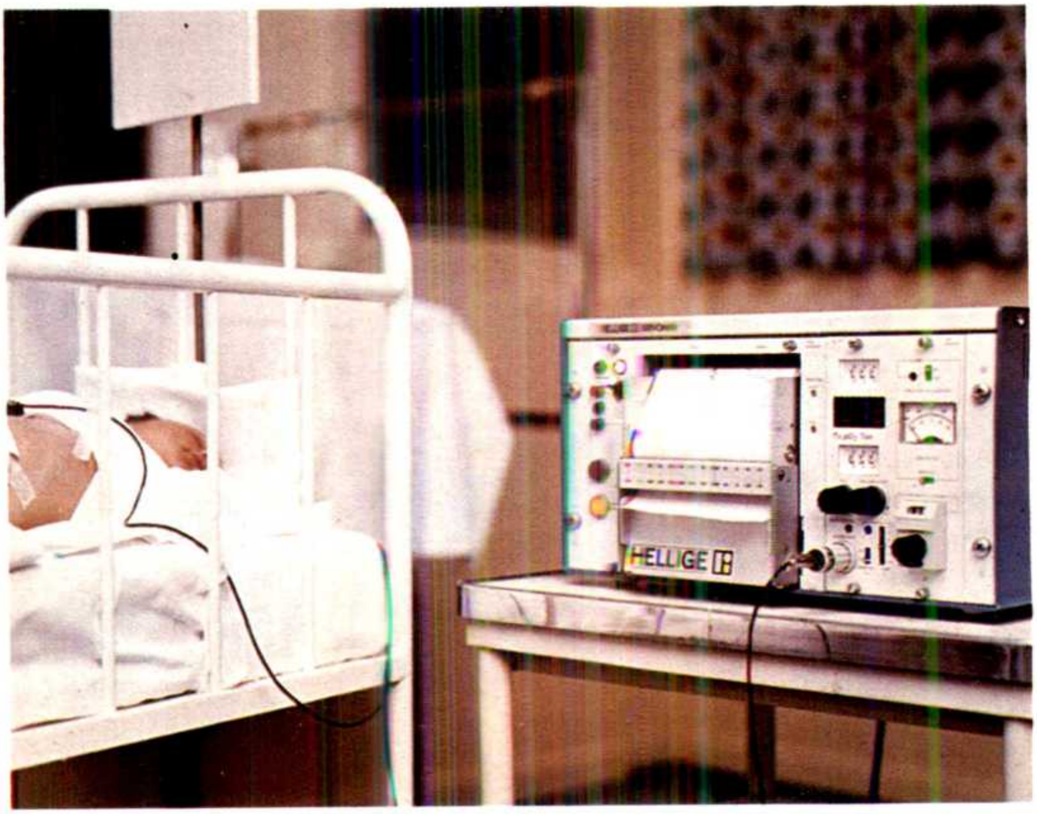

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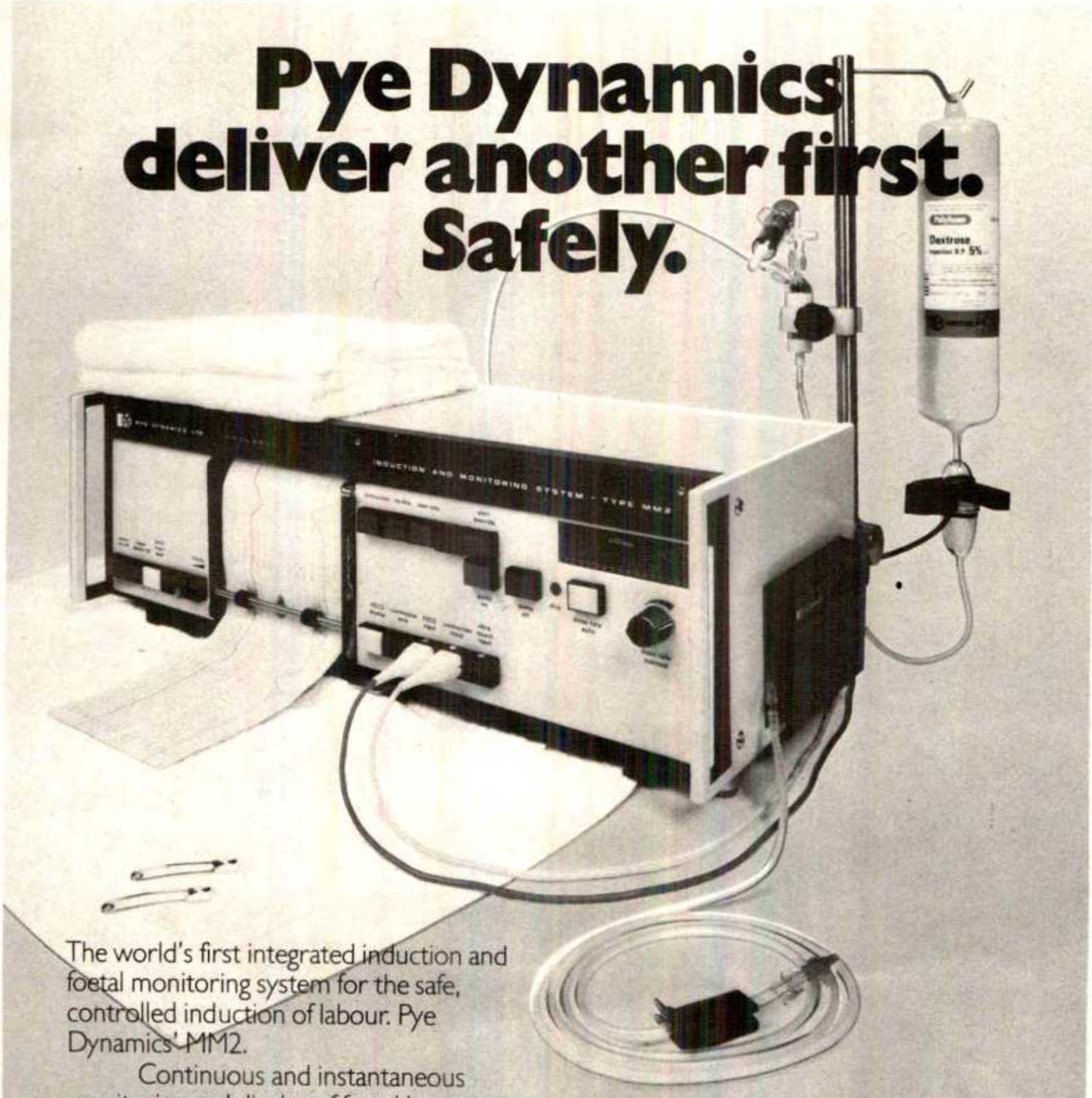

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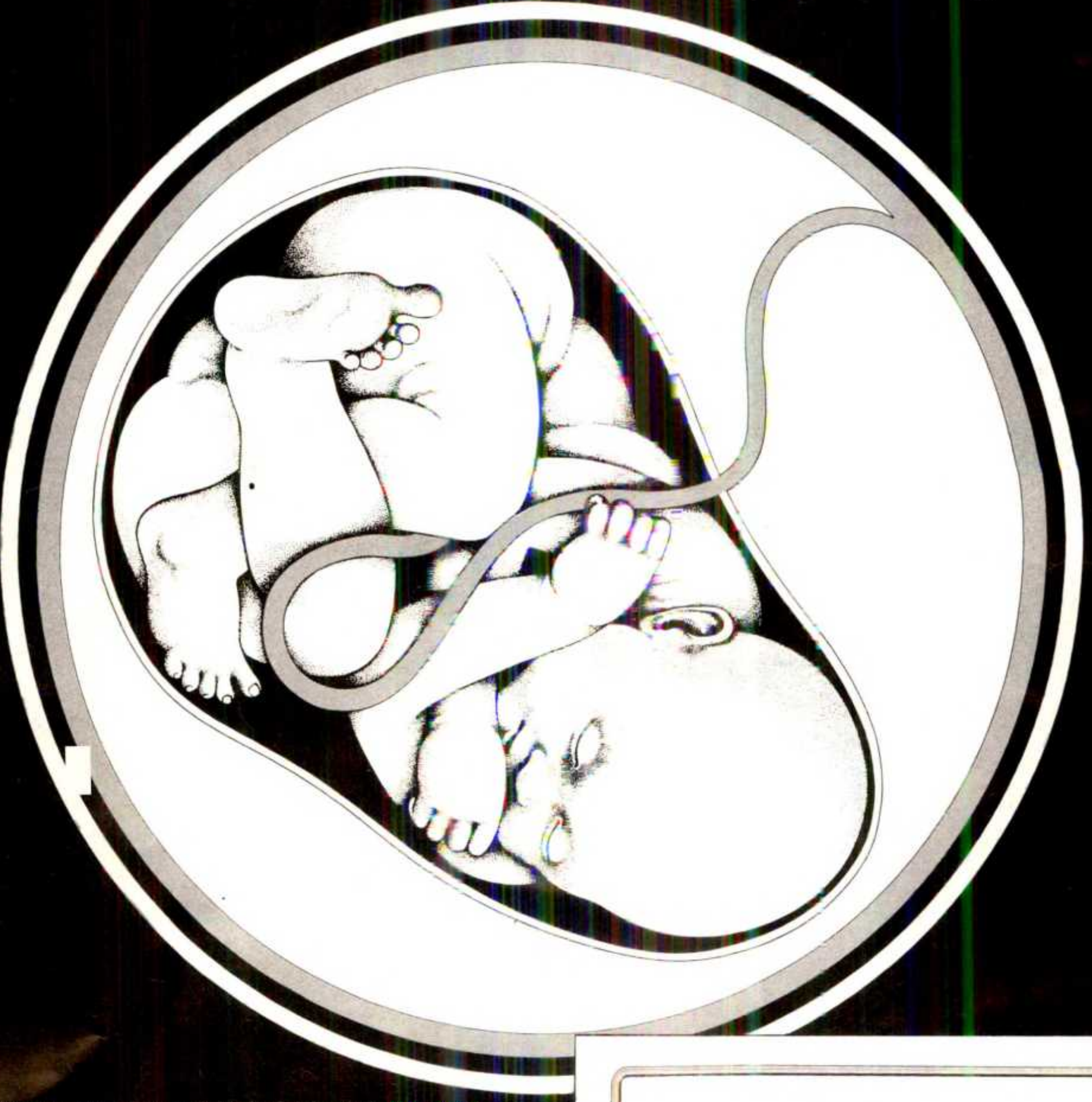

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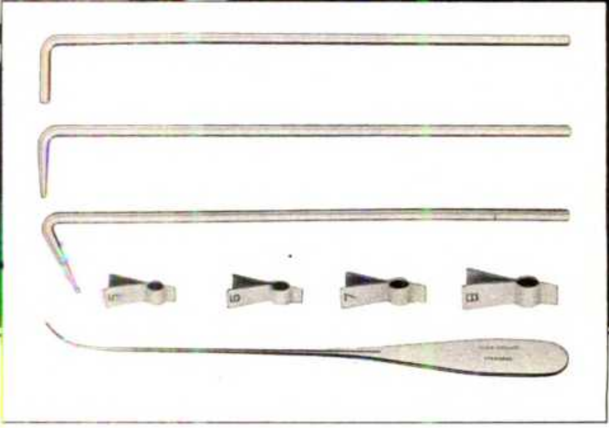

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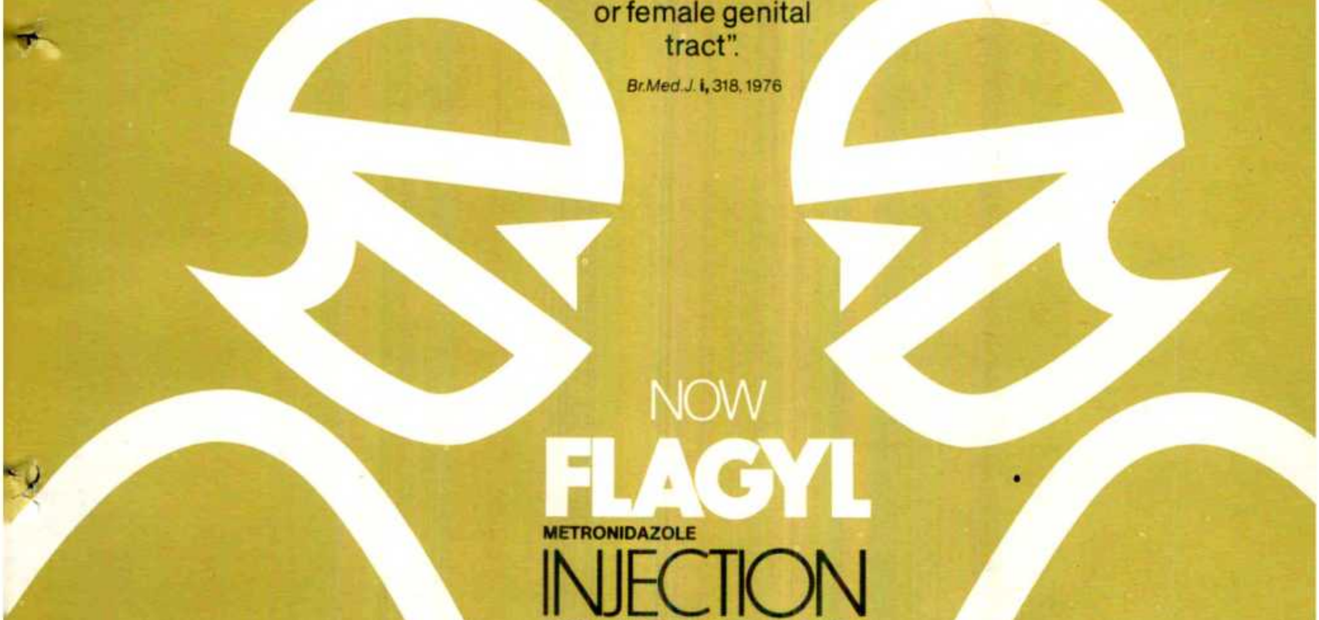

Anaerobic Bacteria

"...the major cause of sepsis after surgery of the gastrointestinal tract or female genital tract".

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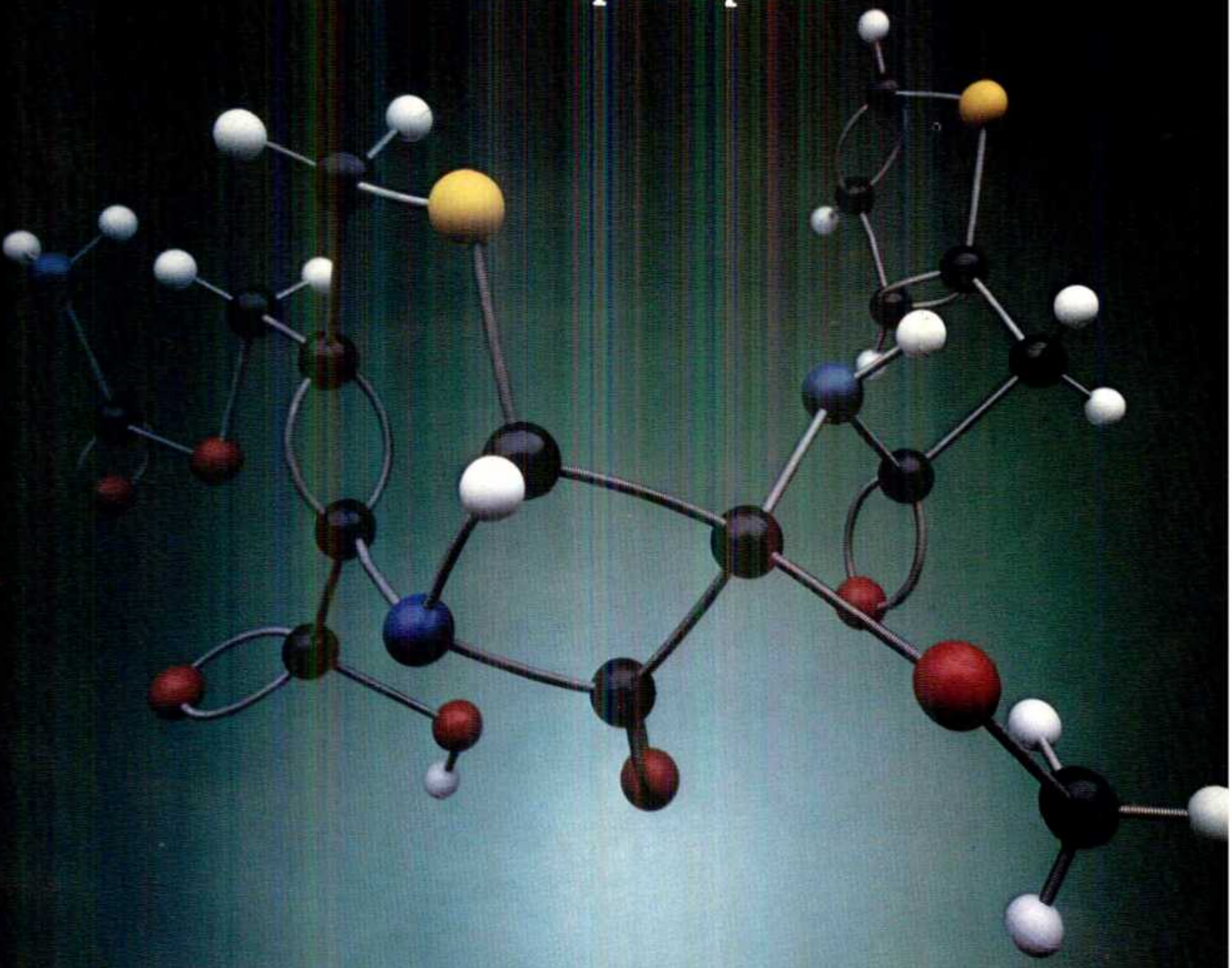

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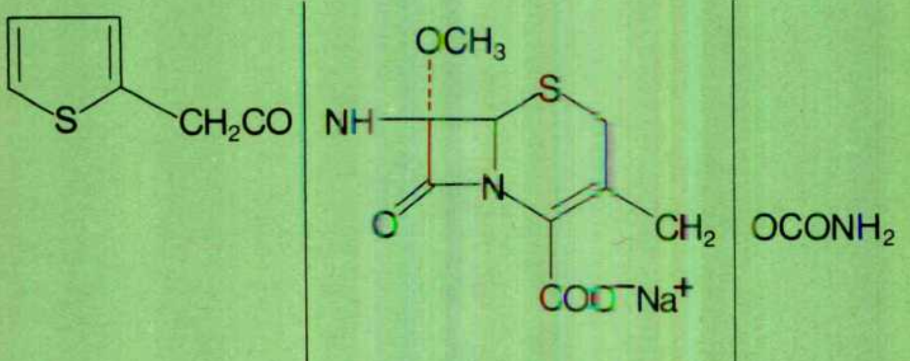

Thienylacetyl side-chain:

enhances activity against Gram-positive pathogens

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provides bactericidal activity against a wide range of pathogens, Gram-negative in particular. 7α-methoxy group protects against β-lactamase degradation

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protects antibiotic from being metabolised

▼Prescribing information

Indications

'Mefoxin' is a broad-spectrum bactericidal antibiotic indicated for the treatment of infections caused by susceptible strains of Gram-positive and Gram-negative pathogens both aerobic and anaerobic.

'Mefoxin' has been clinically effective not only in infections due to antibiotic-sensitive organisms, but also in infections due to organisms resistant to one or more of the following antibacterial agents: penicillin, ampicillin, carbenicillin, tetracyclines, erythromycin, chloramphenicol, cephalosporins, kanamycin, gentamicin, tobramycin, and sulphamethoxazole-trimethoprim.

Many Gram-negative pathogens are resistant to penicillins and cephalosporins through the action of the β-lactamases which are produced by these pathogens. 'Mefoxin' is remarkably stable in the presence of these bacterial β-lactamases, both penicillinases and cephalosporinases. Hence, the clinical efficacy of 'Mefoxin' extends to many infections caused by such pathogens.

'Mefoxin' is indicated for the treatment of the following infections caused by sensitive bacteria:
Peritonitis and other intra-abdominal and intrapelvic infections; female genital tract infections; septicaemia; urinary tract infections; respiratory tract infections; bone and joint infections; and skin and soft-tissue infections.

'Mefoxin' is indicated for adjunctive therapy in the surgical treatment of infections, including abscesses, infection complicating hollow visceral perforations, cutaneous infections, and infections of serous surfaces, whether caused by aerobes, mixed aerobes and anaerobes, or anaerobes.

Microbiology

'Mefoxin' is active *in vitro* against:

Aerobic bacteria

Gram-positive cocci including: Staphylococci (including coagulase-positive, coagulase-negative, and penicillinase-producing strains); Group A beta-haemolytic streptococci *(Streptococcus pyogenes)*; Group B beta-haemolytic streptococci *(Streptococcus agalactiae)*; *Streptococcus pneumoniae (Diplococcus pneumoniae)*; other streptococci (except group D streptococci including

faecalis); *Gram-negative cocci* including: *Neisseria gonorrhoeae* (including penicillinase-producing strains); *Neisseria meningitidis*. *Gram-negative rods (facultative anaerobes)* including: *Escherichia coli*; *Klebsiella pneumoniae*; Klebsiella spp.; *Proteus mirabilis*; Proteus (indole-positive); *Proteus vulgaris*; *Proteus rettgeri*; *Proteus morganii*; *Haemophilus influenzae*; *Serratia marcescens*; Providencia spp.; Salmonella and Shigella spp.

Anaerobic bacteria

Gram-positive cocci *including:* Peptococcus spp.; Peptostreptococcus spp.; Micro-aerophilic streptococcus. *Gram-positive rods including: Clostridium perfringens*; Clostridium spp.; Eubacterium spp.; *Propionibacterium acnes*. *Gram-negative cocci including:* Veillonella spp. Gram-negative rods *including: Bacteroides fragilis*; *Bacteroides melaninogenicus*; Bacteroides spp. (including both penicillin-susceptible and penicillin-resistant strains); Fusobacterium spp.

'Mefoxin' is active against some strains of the following bacteria: *Acinetobacter calcoaceticus* var. *anitratum (Herellea vaginicola)*; *Acinetobacter calcoaceticus* var. *lwoffi (Mima polymorpha)*; *Alcaligenes faecalis*; Citrobacter spp. and Flavobacterium spp.; Enterobacter spp.

'Mefoxin' is not active against Pseudomonas, most strains of enterococci and many strains of *Enterobacter cloacae*.

Dosage and administration

'Mefoxin' may be administered intravenously or intramuscularly. (See reconstitution directions for each route below.) Dosage and route of administration should be determined by severity of infection, susceptibility of the causative organisms, and condition of the patient.

Therapy may be started while awaiting the results of susceptibility testing.

Dosage: The usual adult dosage is 1 g or 2 g of 'Mefoxin' every eight hours. (See chart.)

In adults with renal insufficiency, an initial loading dose of 1 g or 2 g may be given. After a loading dose, the following recommendations

Usual adult dosage

Type of infection	Dose	Frequency	Total daily dosage
Uncomplicated	1 g	Every 8 hours (occasionally every 6 hours)	3 g (4 g)
Moderately severe or severe	2 g	Every 8 hours (occasionally every 6 hours)	6 g (8 g)
Infections generally needing antibiotics in higher dosage	3 g (2 g)	Every 6 hours (Every 4 hours)	12 g

Maintenance dosage of 'Mefoxin' in adults with reduced renal function

Renal function	Creatinine clearance (ml/min)	Dose	Frequency
Mild impairment	50-30	1-2 g	Every 8-12 hours
Moderate impairment	29-10	1-2 g	Every 12-24 hours
Severe impairment	9-5	0.5-1 g	.Every 12-24 hours
Essentially no function	<5	0.5-1 g	Every 24-48 hours

In the patients undergoing haemodialysis, the loading dose of 1 to 2 g should be given after each haemodialysis, and the maintenance dose should be given as indicated in the table above.

Intravenous administration: reconstitute 'Mefoxin' with Water for Injection BP. 1 g is soluble in 2 ml. Although 'Mefoxin' is very soluble, for intravenous use it is preferable to add 10 ml of Water for Injection BP to the 1 g vial or the 2 g vial. Shake to dissolve and then withdraw entire contents of vial into syringe.

Outstanding clinical benefits

Wide-ranging activity against Gram-negative and Gram-positive pathogens, anaerobes as well as aerobes.

Unusually high resistance to β-lactamase degradation.

Highly active against *Bacteroides fragilis* and strains of *E.coli*, Serratia, Klebsiella spp. and Proteus spp. (including indole positive strains) resistant to other antibacterial agents.

Offers high levels of active antibiotic in the body.

A record of relative safety comparable to the penicillins.

Prime indications include:

– Peritonitis, and other intra-abdominal and intrapelvic infections.
– Female genital tract infections
– Septicaemia
– Skin and soft tissue infections
– Bone and joint infections
– Urinary tract infections
– Respiratory tract infections (e.g. inhalation pneumonia)

Specific clinical profile

'Mefoxin' is exceptionally valuable wherever Gram-negative pathogens and anaerobes are involved in mixed infections. When mixed infections are suspected, therapy may be started while awaiting the results of susceptibility testing.

Mefoxin®

Cefoxitin sodium, MSD
INJECTION

First of the cephamycins

the vein over a period of 3 to 5 minutes or may be given through the tubing when the patient is receiving parenteral solutions.

An intermittent intravenous infusion of 'Mefoxin' may be employed when large amounts of fluid are to be given. However during infusion of the solution containing 'Mefoxin' it may be advisable temporarily to discontinue administration of any other infusion solution at the same site (by using an appropriate IV infusion set).

A solution of 'Mefoxin' may also be given by continuous intravenous infusion (see below for compatibility and stability).

Intramuscular administration ONLY
Reconstitute 'Mefoxin' 1 g with 2 ml of Water for Injection BP or 0.5% or 1% lignocaine hydrochloride (without adrenaline) solution. 'Mefoxin' is given by deep injection into a large muscle mass. Avoid injection into a blood vessel.

Compatibility and stability
A solution of 'Mefoxin' in Water for Injection BP may be added to the following solutions: 0.9% Sodium Chloride Injection BP, 5% or 10% Dextrose Injection BP, Dextrose and Sodium Chloride Injection (5%/0.9%, 5%/0.45% or 4.3%/0.18%), Lactated Ringer's Injection USP, 5% Dextrose in Lactated Ringer's Injection, 5% or 10% invert sugar in water, 10% invert sugar in saline solution, 4.2% Sodium Bicarbonate Injection BP, M/6 Sodium Lactate Injection BP.

'Mefoxin' as constituted to 1 g/10 ml with Water for Injection BP, Water for Injection BP preserved with parabens or benzyl alcohol, 0.9% Sodium Chloride Injection BP or 5% Dextrose Injection BP maintains satisfactory potency for 24 hours at room temperature, for 96 hours under refrigeration and for 13 weeks in the frozen state.

'Mefoxin' can be administered concomitantly with such antibiotics as gentamicin, tobramycin, carbenicillin or amikacin. When 'Mefoxin' is administered with another antibiotic they should not be mixed in the same syringe.

Contra-indications
'Mefoxin' is contra-indicated in persons who have shown hypersensitivity to cefoxitin. In the absence of clinical experience 'Mefoxin' should not be administered to patients who have shown

Precautions
There is some clinical and laboratory evidence of partial cross-allergenicity between cephamycins and other β-lactam antibiotics (penicillins and cephalosporins). Severe reactions (including anaphylaxis) have been reported with most β-lactam antibiotics.

Before therapy with 'Mefoxin' careful inquiry should be made concerning previous hypersensitivity reactions to β-lactam antibiotics. 'Mefoxin' should be given cautiously to penicillin-allergic patients.

Any patient who has demonstrated some form of allergy, particularly to drugs, should receive antibiotics cautiously. If an allergic reaction to 'Mefoxin' occurs, the drug should be discontinued.

The total daily dosage should be reduced when 'Mefoxin' is administered to patients with transient or persistent reduction of urinary output due to renal insufficiency (see 'Dosage and administration') because high and prolonged serum antibiotic concentrations can occur from usual doses.

Interference with laboratory tests
A false-positive reaction to glucose in the urine may occur with reducing substances but not with the use of specific glucose oxidase methods.

Use in pregnancy
Use of the drug in women of childbearing potential requires that the anticipated benefits be weighed against possible hazards. Reproductive and teratogenic studies have been performed in mice and rats and have revealed no evidence of impaired fertility or harm to the foetus due to 'Mefoxin'. There are no controlled studies with 'Mefoxin' in pregnant women.

Nursing mothers
'Mefoxin' is excreted in human milk.

Infants and children
Pending the outcome of clinical trials in progress, the conditions for use of 'Mefoxin' in infants and children remain to be defined.

Side effects

stopping treatment and usually have been mild and transient.

Local reactions
Thrombophlebitis has occurred with intravenous administration. Pain, induration and tenderness after intramuscular injections have been reported.

Allergic
Maculopapular rash, urticaria, pruritus, eosinophilia, fever and other allergic reactions have been noted rarely.

Gastro-intestinal
Diarrhoea has been reported rarely.

Blood
Transient leucopenia and neutropenia have been reported. Some individuals, particularly those with azotaemia, may develop positive direct Coombs tests during therapy with 'Mefoxin'.

Liver function
Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase have been reported rarely.

Kidney
Elevations in serum creatinine and/or blood urea levels have been observed. The role of 'Mefoxin' in changes in renal function test is difficult to assess, since factors predisposing to pre-renal azotaemia or to impaired renal function usually have been present.

Presentation
In vials containing 1 g or 2 g of cefoxitin as the sodium salt. Each gram of cefoxitin contains approximately 51.5 mg (2.2 mEq) sodium.

Product licence numbers: 1 g 0025/0130, 2 g 0025/0131

Product authorisation numbers: 1 g PA 35/42/1, 2 g PA 35/42/2

Issued October 1978

Additional information is available to the medical profession on request.

Basic NHS cost is £4.47 for the 1 g vial and £8.94 for the 2 g vial.

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R12

Radiometer's non - invasive oxygen monitor is a bloodless revolution in Po_2.

Transcutaneous Oxygen Measurement

Radiometer's extensive experience in measuring blood oxygen partial pressure has led to the development of an innovative transcutaneous oxygen electrode.

This new electrode is a special design of the polarographic oxygen electrode which, applied on the skin, creates hyperaemia in the dermal capillaries and makes the skin permeable to oxygen so that Po_2 on the skin surface reflects the changes in the oxygen status in the central arteries, thus supplying a continuous measure of the patient's oxygen status, while eliminating the tedious and hazardous procedure of obtaining blood samples.

The importance of this is stressed specifically in neonatal intensive care.

Radiometer's Transcutaneous Electrode

At slightly elevated temperatures, oxygen diffuses readily through the skin, and at the same temperatures the dermal capillaries are hyperaemized to a Po_2 value close to that of the central arteries. The transcutaneous polarographic Po_2 electrode is therefore built together with a heating circuitry controlled by a built-in thermostat which automatically kee
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After a few minutes of temperature st
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with the central arterial Po_2 with a delay
a few seconds only. The Radiometer
Po_2 electrode is a mono-micro catho
electrode fitted with a polypropyle
membrane. It assures an oxygen co
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ing through the skin.

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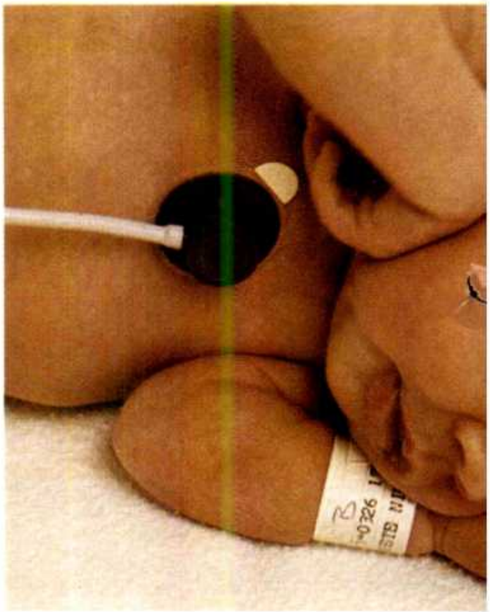
over a large area (32.4 mm^2) from t
heated anode and via a sterile contact
to ensure a homogeneous heat trans
without overheating.

The Radiometer tc-Po_2 electrode
mounted on the skin by means of a



nurse from Radiometer.

parate fixation ring, preventing undue pressure on the membrane. Additional fixation rings can be pre-mounted on the patient so that the electrode can be moved from site to site without delay or recalibration.

These are all constructional details uccesfully serving the purpose of btaining high correlation to aO_2 and avoiding burning of he skin.

Radiometer's
TCM1 tc-Po_2 Monitor

The monitor has three displays or tc-Po_2 (mmHg or kPa), electrode temerature (°C), and electrode heat conumption (mW).

The electrode temperature is adjustable between 35°C and 50°C and can be preset and controlled within 0.1°C. The electrode heat consumption is influenced by blood flow and may be used as an arbitrary measure of blood perfusion.

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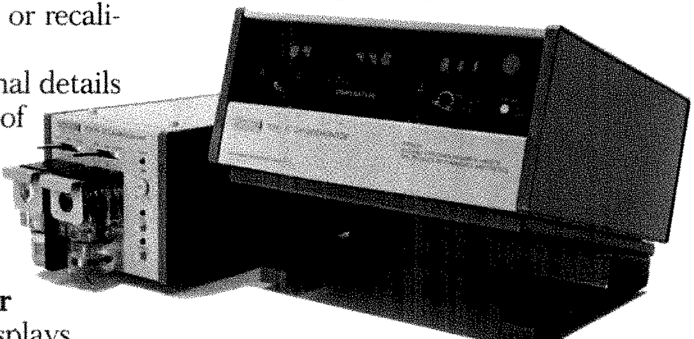
Availability from Radiometer

In order to truly evaluate Radiometer's transcutaneous measuring system, contact us or our local representative to obtain additional information. Reprints, literature and technical material are available upon request.



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controls pituitary gonadotrophins without exerting oestrogenic or progestogenic activity

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Symposium on Danol, London.

1. J. int. med. Res. (1977) 5 (Suppl. 3): 86-91. **2.** ibid., 101-108. **3.** ibid., 98-100. **4.** ibid., 114-123. **5.** ibid., 109-113.

WINTHROP

Danol is a trade mark. Full prescribing information on request from Winthrop Laboratories, Surbiton-upon-Thames, Surrey.

Prescribing Information

Presentation
White egg-shaped pessaries each containing 150mg econazole nitrate and melting at 37°C.

Uses
Vaginitis due to Candida albicans and other yeasts.

Dosage and Administration
One pessary should be inserted high into the vagina each evening for three consecutive days.

Contra-indications, Warnings etc.
Contra-indications: None known.
Side-effects: None known.
Overdosage: Not applicable.
Precautions: Hypersensitivity has never been recorded, but if it should occur, administration of the product should be discontinued.
Gyno-Pevaryl* 150 vaginal pessaries are effective in the treatment of vaginal candidiasis associated with pregnancy but as with any new product, caution should be exercised when prescribing during the first trimester.

Pharmaceutical Precautions
Store in a cool place.

Legal Category
Prescription only (POM).

Package Quantities
Each pack contains 3 pessaries and a patient instruction leaflet. The pessaries are individually sealed in a white plastic strip marked **Gyno-Pevaryl*** 150 at regular intervals.

Further Information
Anogenital hygiene is important to help prevent re-infection.
PL0076/0058

Name and address of Licensee
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Further information and data sheet available on request.

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International Symposium on "ADRENAL ANDROGENS"

University of Siena, Italy, 7th–9th October 1979.

Scientific Committee: A. R. Genazzani (Siena, Italy), G. Giusti (Florence, Italy), P. C. MacDonald (Dallas, USA), W. D. Odell (Torrance, USA), P. K. Siiteri (San Francisco, USA), J. H. H. Thijssen (Utrecht, Holland), A. Vermeulen (Ghent, Belgium).

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Under the auspices of the Italian Society of Obstetrics and Gynaecology, the Italian Society of Endocrinology, the Italian Society of Fertility and Sterility and the C.N.R. project "Biology of Reproduction".

Topics

Androgen secretion by human adrenal and testis cells in culture.
Hormonal factors controlling and stimulating adrenal androgen secretion.
Adrenal androgen metabolism and conversion in humans.
Adrenal androgens in pregnancy, in prepuberty, puberty, adult life, ageing, and in postmenopausal women, hirsute and obese subjects.
Enzymatic adrenal defects.
Adrenal androgens and endometrial cancer.
Adrenal androgen effects on bone metabolism and on the hypothalmus-pituitary-gonadal axis.

Round Table: Adrenal androgens—the future.

Speakers:

G. Abraham (Rolling Hills, USA)
D. Anderson (Manchester, England)
A. Genazzani (Siena, Italy)
G. Giusti (Florence, Italy)
P. MacDonald (Dallas, USA)
A Neville (Sutton, England)
B. Nordin (Leeds, England)
W. Odell (Torrance, USA)
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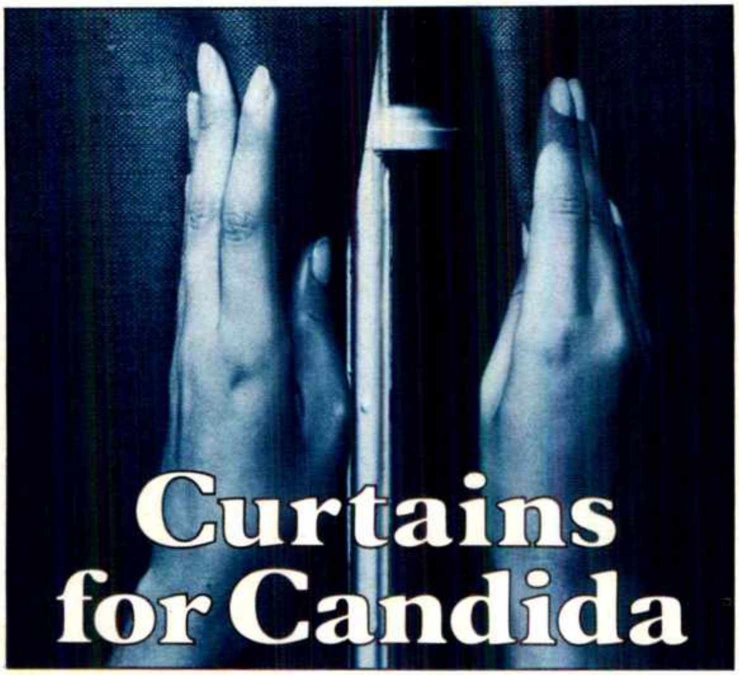
J. Poortman (Utrecht, Holland)
M. Serio (Florence, Italy)
P. Siiteri (San Francisco, USA)
P. Sizonenko (Geneva, Switzerland)
J. Thijssen (Utrecht, Holland)
D. Tulchinsky (Boston, USA)
A. Vermeulen (Ghent, Belgium)

Official language: English

Researchers are invited to submit 250 word abstracts of unpublished work for oral or poster presentation before 15th June 1979. A limited number of free communications will be selected for presentation and published with the invited lectures in the proceedings. Abstracts of invited lectures, free communications and posters will be published in a separate volume.

Scientific Secretary: Prof. A. R. Genazzani,
Centro di Fisiopatologia della Riproduzione,
Università di Siena,
Via Paolo Mascagni, 46
53100 SIENA, Italy.
Tel. 0577/49206—44051.



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Ecostatin also ensures
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Ecostatin is also available as a cream for a wide range of dermatomycoses.

Prescribing Information
PRESENTATION
White, opaque oval pessaries each containing 150mg econazole nitrate in an hydrogenated vegetable oil base.
USES
Actions Econazole nitrate has a broad spectrum of antifungal activity. It is highly active against Candida albicans and other Candida species and is effective in controlling infections of the vagina and vulva caused by such organisms (thrush).
Indications Vulvovaginal candidosis.
DOSAGE AND ADMINISTRATION
One pessary to be inserted at bedtime for 3 consecutive nights.
Administration should be continued even if menstruation occurs, and despite the disappearance of signs and symptoms of the infection.
The pessary should be inserted high into the vagina while the patient is supine. Although a three day course of therapy usually suffices it may be necessary to institute a second course of therapy.
CONTRA-INDICATIONS, WARNINGS, etc.
Contra-indications None known.
Precautions Ecostatin pessaries are effective in the candidal vaginitis associated with pregnancy. However, as with other agents, it should not be used during the first trimester of pregnancy unless the physician deems it's use essential for the welfare of the patient.
Side Effects Very occasionally patients may complain of discomfort; this is usually transitory and disappears with continued treatment. Rarely is it necessary to discontinue econazole pessary treatment.
PHARMACEUTICAL PRECAUTIONS
Storage Store at room temperature.
LEGAL CATEGORY POM
PACKAGE QUANTITIES Packs of three pessaries with applicator.
PRODUCT LICENCE NUMBER, NAMES AND ADDRESSES
Product Licence Number 0085/0013.
References
1. Avram, A. et Grupper, Ch. Bulletin de la Société Française de Mycologie Médicale 1975 Tome IV (2).
2. Preusser, H.J. et al. Research Report 1974. On file Cilag-Chemie.
3. Schweisfurth, R. Research Report 1974. On file Cilag-Chemie.

Ecostatin is a registered trade mark for econazole nitrate and FAIR Laboratories is the registered user.
Further information is available from:
FAIR Laboratories,
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Reversibility of Female Sterilization

edited by I. Brosens and R. Winston

November/December 1978 x + 196pp., £7.50

0.12.136080.6 (Academic Press) 0.8089.1150.3 (Grune & Stratton)

Sterilization has become the most extensively used method of contraception, and at the same time the demand for reversal of sterilization has also increased. In view of this increasing demand, a number of leading specialists in the field were invited to discuss different aspects of potential reversibility of female sterilization at the first international symposium on the subject, held in Leuven, Belgium, in June 1977. This book comprises both the papers presented and the discussions held at the symposium. Although dealing mainly with technical aspects of reversal, the papers also include discussions on the scientific background to fallopian tube function (physiology, pathology and ultra-structure), different methods of sterilization with particular reference to their reversibility, and psychological and sociological aspects of reversal and the demand for it. As more women seek reversal of sterilization, increasing numbers of gynaecologists and family planning doctors and counsellors will be faced with the need to know the real possibilities of reversal. For them this book will be invaluable, with its extensive discussions representing the current opinions of leading experts in this field, most of which have not been previously published.

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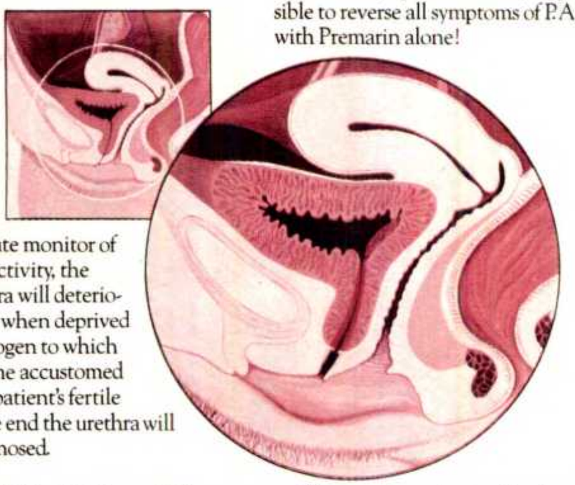
The Diagnosis and Treatment of P.A.U.

Post-menopausal Atrophic Urethritis

Older women presenting with frequency, urgency and painful micturition often describe their condition as 'cystitis.' There can hardly be a woman in existence who has not suffered the transient discomfort of a 'simple cystitis' at some time in her early life, but as she reaches middle age, although the symptoms may appear to her as the same, the cause, particularly in the absence of infection, may be atrophic changes in the distal urethra responding to hormonal disparities of the menopause.[1]

Untreated, these atrophic changes can be devastating and demoralising in their effect.[2]



A delicate monitor of oestrogen activity, the distal urethra will deteriorate rapidly when deprived of the oestrogen to which it has become accustomed during the patient's fertile years. In the end the urethra will become stenosed.

Treating P.A.U. with Premarin*

Treatment with Premarin 0.625mg tablets can effect a rapid and complete elimination of symptoms.[3]

1. Oestrogen deprivation can be confirmed by vaginal cytology or assumed in the presence of vaginal atrophy or dryness, or other symptoms of oestrogen lack.

2. Low-dose Premarin (0.625mg) administered cyclically – 21 days on, 7 days off – for 3 months. Urethral epithelium is remarkably sensitive to oestrogens and so will respond to this low dose.

3. Frank urinary tract infection should of course be treated with appropriate antibiotics or urinary anti-infectives.

4. If treatment is instigated in the early stages of this disorder – for instance within the first six/twelve months of symptoms – it may be possible to reverse all symptoms of P.A.U. with Premarin alone!

However in patients with a longer history of frequency and urgency, if oestrogen therapy does not effect a rapid and complete elimination of symptoms then the possibility of a secondary urethral stenosis should be considered.

References:

1. Willington, F. L. The Practitioner, 220, 739, 1978.
2. Smith, P. J. B., Averst Medical Library Vol. 2, No 2, 1978.
3. Smith, P. J. B., The Practitioner, 218, 97, 1977.

PREMARIN

(conjugated oestrogens)

Premarin tablets

Uses

Menopausal and post-menopausal oestrogen replacement therapy and allied disorders such as post-menopausal osteoporosis, atrophic vaginitis and post-menopausal atrophic urethritis.

Dosage and Administration

Menopausal and post-menopausal oestrogen deficiency: Premarin 0.625–1.25mg daily is the usual starting dose. Cyclic administration is recommended (three weeks' dosage followed by one week's rest). For maintenance, the lowest effective dose should be used. It is recommended that a physical examination, including endometrial assessment, whenever possible be performed at the time of the initial examination, and the endometrium assessed regularly thereafter at 6–12 month intervals. Break through bleeding is an indication for further investigation.

Contra-Indications: Uterine myomatosis. Endometriosis Cancer of the breast and uterus. Active hepatic, renal and severe cardiac disease. Undiagnosed genital bleeding. Diabetes. Thyrotoxicosis. Pregnancy. Personal history of thrombosis.

Precautions

In the female – prolonged use of unopposed oestrogen may increase the risk of endometrial carcinoma.

Side Effects

Premarin is unlikely to cause gastro-intestinal symptoms, headaches, breast tenderness, salt and water retention which can occur with other forms of oestrogen therapy.

Product licence numbers

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21 tablet pack £0.80.
Premarin Tablets 1.25mg. PL 0607/5001.
21 tablet pack £1.30.
Premarin Tablets 2.5mg. PL0607/0002.
30 tablets £2.40.

Ayerst International

Full prescribing information:
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Telephone: Farnborough (Hants) (STD 0252) 511981.

*Premarin is a Registered Trade Mark.

P, 25, 398

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Therapy was well tolerated with the most common side effects, nausea and local tissue reaction, occurring in less than 30% of the women. No adverse foetal effects were noted.

Prostin E2 exerts no antidiuretic effect and, unlike oxytocin, has caused no higher incidence of neonatal hyperbilirubinaemia than that encountered with normal labour.

Therapy is easily administered according to an uncomplicated, step-wise dosage schedule.

Availability

As dinoprostone, 1 mg/ml, in ethanol, in 0.75 ml. ampoules.

Prostin E2 is available for use only in hospitals and clinics with specialised obstetric and gynaecology units.

Full product information available on request.

Product Licence Number 0032/0020

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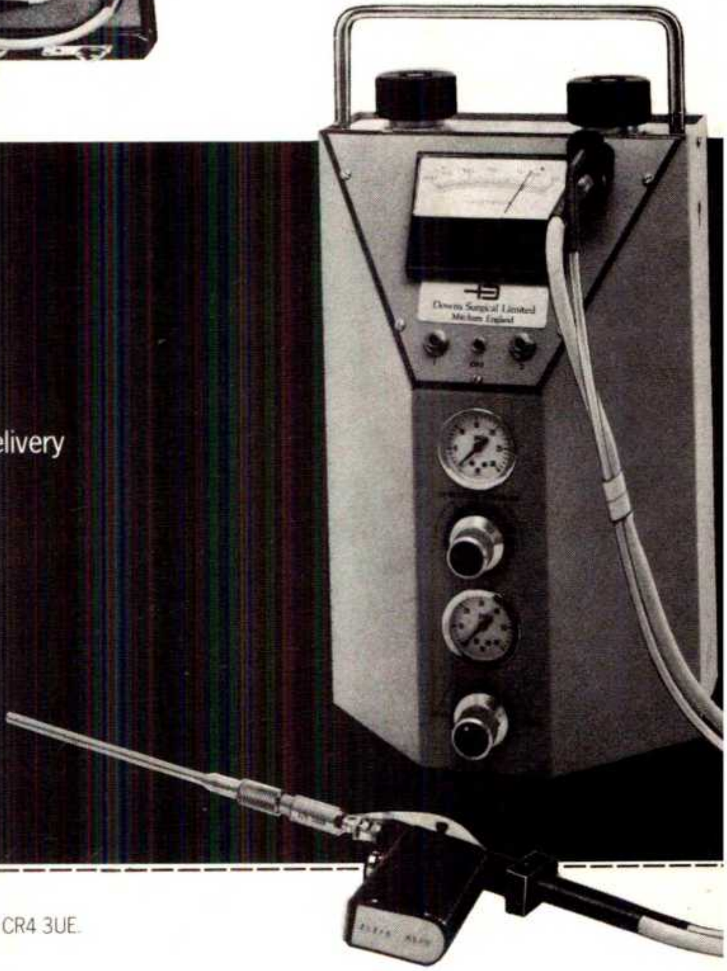

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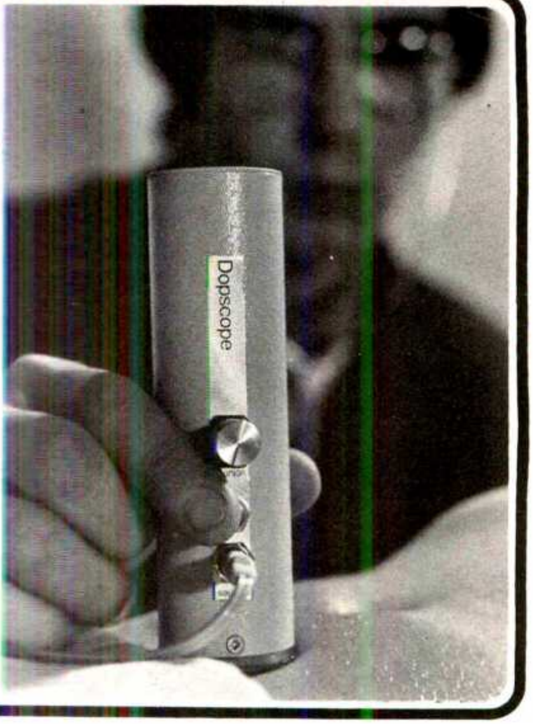

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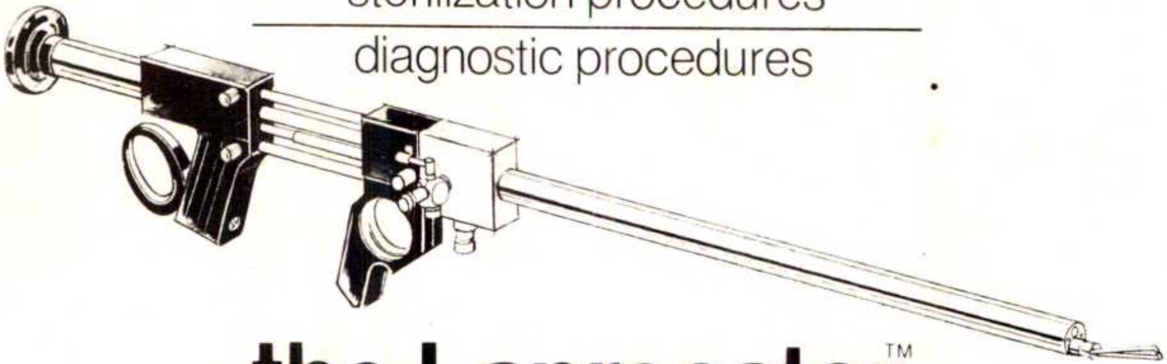

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*316/1-2 Breast Feeding ☐
328 Development of the Fetus ☐
352/1-2 Labour and Delivery ☐
358/1-2 Pre-Term Infant ☐
362/1-2 Lower Segment Caesarian Section ☐
368 Le Boyer Technique ☐
370/1-2 Birth a Shared Experience ☐

Slidesets ☐ Filmstrips ☐ Cassetted Tapes ☐ *Filmloops ☐ *16mm Films ☐ Equipment ☐ Also Literature ☐

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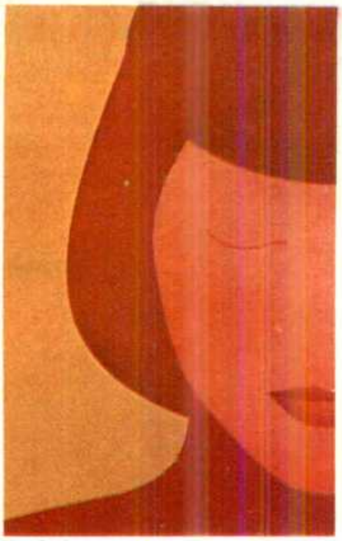
BJOG

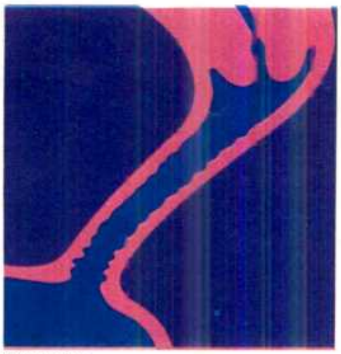


Hot flushes, sweating

Irritability, anxiety, depression

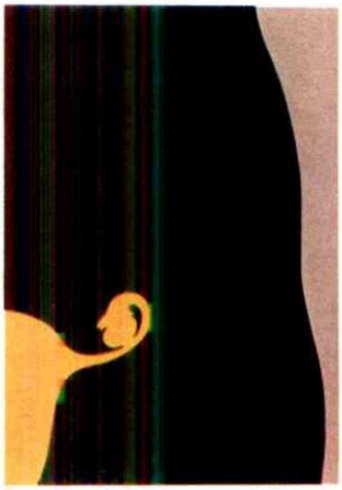
Endometrial hyperplasia

Below the surface of the climacteric



Dry vagina

Osteoporosis (affects one patient in three[1])



Irregular periods

Cyclo-Progynova

The all-round treatment for menopausal problems

Oestrogen with progestogen to protect against endometrial hyperplasia

1. *Modern Medicine* 45, 10, 1977 **Presentation** Circular memo-pack holding 11 white tablets each containing 2 mg oestradiol valerate and 10 orange tablets each containing 2 mg oestradiol valerate and 0.5 mg norgestrel. All tablets are sugar-coated with 'B' i regular hexagon printed on each side. **Uses** i) The alleviation of symptoms characteristic of the menopause. Cyclo-Progynova may be used both before and after the menopause. ii) The correction of irregularities of the menstrual cycle caused by abnormal oestrog production and diminishing corpus luteum function as the menopause approaches. N.B. Cyclo-Progynova should not be used as an oral contraceptive. **Dosages and administration** Patients still having periods may start tablet-taking on the fifth day of t cycle, counting the first day of bleeding as day one. Patients whose periods are very irregular or non-existent may start taking the tablets at any time. The memo-pack sticker should be attached so that the starting day is correctly indicated in the red section. O white tablet is taken daily for eleven days, followed by one orange tablet daily for ten days. An interval of seven days follows each pack during which bleeding will normally occur. **Contra-indications** Severe disturbances of liver function, jaundice or persist itching during a previous pregnancy. Dubin-Johnson syndrome. Rotor syndrome, existing or previous thromboembolic processes (including strokes), sickle-cell anaemia, existing or treated cancer of the breast or endometrium, endometriosis, mastopat myomatous uterus, congenital disturbances of lipid metabolism, a history of herpes of pregnancy, otosclerosis with deterioration in previous pregnancies. **Warnings/Side effects** Use with caution in patients who have a history of congenital abnormalities lipid metabolism. Some women are predisposed to cholestasis during steroid therapy. Uterine fibroids may be affected by oestrogens and should be checked frequently. Note: Treatment should be stopped at once if jaundice or pregnancy occurs or if there i significant rise in blood pressure. **Precautions and special information** Periodical gynaecological examinations are advised for patients receiving long-term treatment with Cyclo-Progynova. In patients with chronic liver disea liver function should be checked every 8–12 weeks. Irregular bleeding during tablet-taking should be investigated. The use of Cyclo-Progynova with an oral contraceptive is not recommended. **Overdosage** Toxic effects of overdos have not been reported, but, if desired, gastric lavage can safely be used. There are no special antidotes, and further treatment should be symptomatic. **Legal category** POM. **Basic NHS price** £2.24. **Product licence number** 0053/00 Further information on Cyclo-Progynova is available on request. **Schering Chemicals Limited**, Pharmaceutical Division, Burgess Hill, West Sussex RH15 9NE.

Schering

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ACTIVITY AND CHARACTERIZATION OF A LOW MOLECULAR FRACTION PRESENT IN HUMAN AMNIOTIC FLUID WITH BROAD SPECTRUM ANTIBACTERIAL ACTIVITY

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Summary

The isolation of a low molecular weight fraction from amniotic fluid with broad spectrum antimicrobial activity is reported. This fraction consists of a family of peptides whose bactericidal activity is zinc dependent and can be inhibited by phosphate. Peptides are present in early gestation in low concentration increasing in amount from about 28 weeks gestation when antimicrobial activity becomes detectable in whole amniotic fluid. A simple bacteriological assay for this material is described. Its relation to the occurrence of perinatal infection is discussed.

RECENT studies have confirmed that intra-uterine infection remains a significant aetiological factor in both perinatal morbidity and mortality. Antenatal infection can occur with intact amniotic membranes at the onset of labour (Prevedourakis and Papadimitriou, 1970). There is a difference in incidence between countries with varying socio-economic and environmental conditions. The U.S. collaborative mortality survey reported an incidence of 17 per cent in the USA (Naeye, 1977), as compared with a figure of 33 per cent in Ethiopia (Naeye *et al*, 1977).

The mechanisms which help to maintain a sterile environment for the fetus *in utero* are varied. Adaptive and non-specific immunity provide a quarantine system, reducing the opportunity for transplacental infection. Organisms can invade the fetus via the placenta or the lower genital tract. Although the fetus gradually acquires defense mechanisms of its own, they give less than adequate protection. Amniotic fluid is known to exhibit antimicrobial activity; it contains lysozyme, transferrin and a low concentration of immunoglobulin. However, the antimicrobial activity is dependent on adequate levels of zinc in the absence of high levels of phosphate, for hitherto unknown reasons.

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Schlievert *et al* (1976) published a description of a low molecular weight polypeptide in amniotic fluid, with potent bactericidal activity, dependent on the zinc to phosphate ratio, and they examined some of the chemical and biological properties of this compound. We have attempted to repeat this work and confirmed the presence of a zinc dependent antibacterial substance in amniotic fluid. However, we have found substantial differences in both identification of active fractions and in the composition of the antibacterial polypeptide.

MATERIALS AND METHODS

Samples of amniotic fluid were obtained at the time of induction of labour by hindwater rupture and in the second trimester at the time of intra-amniotic prostaglandin termination of pregnancy. Patients receiving antibiotics and samples contaminated with blood or meconium were excluded from the study. Pooled samples of amniotic fluid obtained from several patients at the same gestational age were centrifuged at 3500 rpm for 20 minutes. The supernatants were then passed through a 0·45 μ millipore filter to sterilize the samples if the specimens were not to be fractionated immediately.

Fractionation of amniotic fluid

Substances of molecular weight greater than 2000 daltons were removed by sequential pressure dialysis using an Amicon filter with a PM 10 followed by UM2 membrane. UM2 filtrates were then concentrated by lyophilization. Both filtrates were tested for antibacterial activity.

A 500 mg amount of freeze-dried UM2 filtrate was mixed with 5 ml distilled water and insoluble material removed by centrifugation. The supernatant was then fractionated on Sephadex G10 (Pharmacia) previously equilibrated with distilled water, at a flow rate of 30 ml/hour. Effluent material was collected in 3 ml volumes and monitored for absorption at 280 nm. The pooled fraction of each 280 nm absorption peak was concentrated by lyophilization. Amounts of 100 mg of material from peaks shown to have antibacterial activity (PK2–PK3) were reconstituted to 2 ml with buffer and further fractionated on diethylaminoethyl cellulose (DEAE, Whatman), equilibrated with 0·01 M ammonium formate, pH 8·5. After collection of an exclusion fraction, a buffer gradient 0·01–2 M ammonium formate was run, at a flow rate of 7 ml/hour. Effluent material was collected in 3 ml fractions and monitored for absorption at 280 nm. The peaks observed were lyophilized and dissolved in 2 ml sterile water and assayed for antibacterial activity.

Fractions found to have antimicrobial activity were scanned for spectrophotometric absorption between 18 nm and 450 nm for comparison with previous reported data (Schlievert *et al*, 1976).

Bacterial assay

A solution of double strength phosphate free broth (nutrient broth No. 2 Oxoid CM67) was prepared. One ml of the test material was added to an equivalent volume of double strength broth. For the control, 1 ml of the double strength broth was added to 1 ml of physiological saline. To each test and control an inoculum of approximately 300 colony forming units was added. The bacteria used were Oxford *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), and Group B haemolytic streptococcus (clinical isolate). After 24 hours incubation, bacterial growth was assayed by colony counts, modified from the method of Miles and Misra (1938) or by light absorption at 700 nm. Two controls were used: (i) 1 ml of double strength broth plus 1 ml water without an inoculum of bacteria, and (ii) with bacteria. This technique cannot strictly distinguish between bacteriocidal and bacteriostatic activity. To test the fractions from G10 and DEAE, a solution of zinc chloride was added to ensure a physiological concentration of 0·3 μg/ml. This concentration of zinc was added to the controls.

Phosphate and zinc content

Fractions from the G10 column with antibacterial activity were analysed for their phosphate (Technicon SMA 12/60 Method) and zinc concentration by atomic absorption (I.L. 251).

Stability of the compound

When whole amniotic fluid or biologically active fractions were kept for more than three weeks, even in a sterile environment, antimicrobial activity was lost. An experiment was done to examine the hypothesis that the presence of oxygen and/or light might be responsible for the breakdown of the material. Samples were kept for one month under the following conditions: (1) at 4°C, (2) at room temperature in light and (3) in vacuum at 4°C in the dark. These samples were examined by thin layer chromatography (TLC) using butanol/acetic acid as the solvent and stained with ninhydrin.

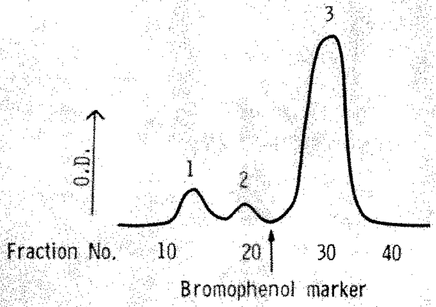

FIG. 1

Fractionation of freeze-dried UM2 filtrate on G10 Sephadex monitoring for absorption at 280 nm.

RESULTS

Fractionation of amniotic fluid and bacterial assay

The elution profile during G10 fractionation is shown in Figure 1. Three peaks were eluted with absorption at 280 nm. Peak 1 eluted between tubes 9 and 17, peak 2 between tubes 18 and 22, peak 3 between 23 and 41. Bromophenol marker, molecular weight 670 daltons, eluted between peaks 2 and 3. Chemical characteristics of peaks 2 and 3 are shown in Table I. The yield of peak 3 material was approximately 1·2 gm/l of amniotic fluid. As peak 1 did not demonstrate antibacterial activity, it was not subjected to further analysis.

Figures 2 and 3 show the elution profiles seen after fractionation on DEAE of (G10) peaks 2 and 3 respectively. The first two peaks were incompletely separated and eluted between tubes 9 and 15. The third peak in Figure 2, eluted between tubes 72 and 75 whereas in Figure 3, the

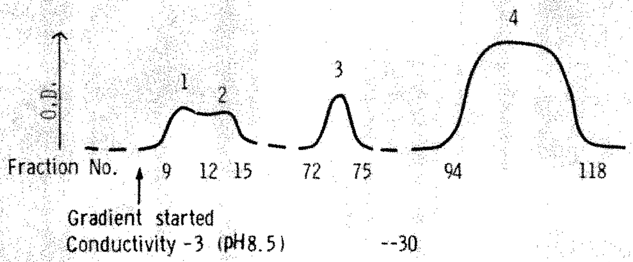

FIG. 2

Fractionation of peak 2 (from G10) on DEAE monitoring for absorption of 280 nm.

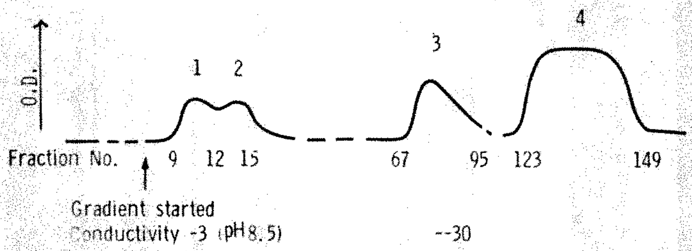

FIG. 3

Fractionation of peak 3 (from G10) on DEAE monitoring for absorption at 280 nm.

third peak was more diffuse eluting between tubes 67 and 95. The fourth peak in Figure 3 was shown to elute later than in Figure 2 but was of equal dimensions.

The yield of active material from DEAE was approximately 50 per cent by weight in both cases. When the four peaks from DEAE were run on TLC using the solvent described, only peaks 1 and 2 stained with ninhydrin. Peak 4 was fluorescent under ultraviolet light and its appearance on TLC was similar to that of a xanthine derivative. Peak 4 material also absorbed strongly at 280 nm. Table II shows the antimicrobial activity of whole amniotic fluid and a number of fractions. Whole amniotic fluid PM 10, and UM2 filtrates were bactericidal and Peak 1 (from DEAE) varied in its activity. Bacteridical fractions were equally effective against *Staphylococcus aureus*, haemolytic streptococcus, and *E. coli*. Table III displays bacterial colony counts following 24 hours incubation with test material. The minimum inhibitory concentration of G10 peak 3 was 500 μg/ml. Below this concentration, using the technique described, no antibacterial activity was seen.

TABLE I

Phosphate and zinc content of the three peaks from G10

	Peak 1	Peak 2	Peak 3
Phosphate (mmol/l)	0·11	0·03	0·01
Zinc (μmol/l)	27·8	14·1	4·3
Approximate phosphate:zinc ratio	4	2	2

TABLE II

Antimicrobial activity of different fractions

Fraction	No activity	Bacteriostatic	Bactericidal
Whole amniotic fluid	—	—	+
PM 10 filtrate	—	—	+
UM2 filtrate	—	—	+
Peak 1 (G10)	+	—	—
Peak 2 (G10)	—	+	—
Peak 3 (G10)	—	—	+
Peak 1 (DEAE)	—	variable	variable
Peak 2 (DEAE)	—	—	+
Peak 3 (DEAE)	—	—	—
Peak 4 (DEAE)	—	—	—

+ no activity: bacterial growth same as control
— no activity: bacterial growth differs from control
Samples were recorded as bacteriostatic where bacterial growth was absent or less than in the control and bactericidal when the count had fallen during incubation.

TABLE III

Antibacterial activity of amniotic fluid and peak 3 from G10 fractionation using Staphylococcus aureus *as test bacteria**

	Test (Colony count)	Control (Colony count)
Whole amniotic fluid	23	390×10^6
2 mg/ml of peak 3	0	300×10^6

Figures are colony counts/0·02 ml.
* The colony counts were made after 24 hours incubation, the inoculum consisted of 300-colony forming units.

Amniotic fluid and gestation

Whole amniotic fluid was shown to have antibacterial activity from 28 weeks gestation. When inactive amniotic fluid obtained at 16 weeks gestation was examined, a similar elution profile from G10 was obtained, and it had a similar appearance on spectrophotometric scanning. However, the concentration of the peaks was less than fractionated term amniotic fluid, as determined by the appearance on scanning and the yield after lyophilization.

Stability of the compound

Table III shows the activity of (G10) peak 3 material stored under different conditions and demonstrates that light and air caused the material to break down. Figure 4 shows the appearance of active and inactive material on TLC. Loss of antimicrobial activity was related

TABLE IV

Test for compound stability with different methods of storage used for one month

Conditions	Activity
Room temperature + light	inactive
4°C + light	inactive
Vacuum + light + 4°C	active
Vacuum + dark + 4°C	active

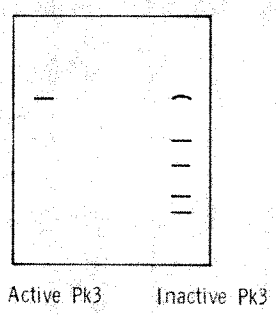

FIG. 4

Thin-layer chromatography (TLC) of active and inactive peak 3 (from G10) stained with Ninhydrin. The inactive material shows the presence of free amino acids.

to the hydrolysis of the compound with the presence of free amino acids. This was confirmed by amino acid analysis.

DISCUSSION

There have been several reports in the literature of the antimicrobial activity of amniotic fluid (Cattaneo, 1949; Galask and Synder, 1970; Miller and Michel, 1976) but only one previous study to examine the nature of the antibacterial material (Schlievert *et al*, 1976). Our observations have confirmed the presence of a low molecular weight fraction with zinc dependent bactericidal activity but do not support the contention that this activity is the property of a single polypeptide chain.

Our fractionation procedure differs significantly from that described by Schlievert *et al* (1976). The initial pressure filtration produced a filtrate with solvents of less than 2000 daltons molecular weight as compared to 10 000 daltons. This fraction was anslysed on G10 Sephadex rather than Biogel P_2 and revealed 3 peaks with absorption at 280 nm as compared to 2 peaks. Finally, our DEAE fractionation employed ammonium formate buffer. Schlievert *et al* (1976) used no buffer during DEAE fractionation and eluted with a sodium chloride gradient. Only one peak eluted, and they then had the problem of desalting a low molecular weight polypeptide. With our technique, two similar polypeptide peaks and two non-protein peaks were eluted. The fourth peak absorbed strongly at 280 nm. This peak probably explains the spectrophotometric characteristics of the G10 fraction, since our preliminary findings on amino acid analysis do not reveal a significant concentration of aromatic amino acids. The substance behaves on chromatography as a xanthine derivative.

In this study, we have used a simple bacteriological assay. The results can be read in terms of bacterial colony counts or by comparison of the transmission of light at 700 nm.

Preliminary amino acids analysis of *this compound* indicates the presence of a family of peptides linked to zinc and possessing a different amino acid content to the active polypeptide described by Schlievert *et al* (1976). However, their single chain could be part of our family of polypeptides. In a future communication, we shall report the amino acid content and structure of the antibacterial compound. We intend to synthesize the active polypeptide(s) with a view to both internal radio labelling and the development of a suitable immunoassay in order to be able to examine its clinical role. A relative deficiency in amniotic fluid of the defence mechanism described here could increase susceptibility to antenatal intrauterine infection, chorioamniotis and premature rupture of membranes.

Perinatal infection caused by haemolytic streptococcus (group B) is a relatively uncommon occurrence compared to the frequency of isolation of this organism on vaginal swabs. This polypeptide may alter the flora of the birth canal. A relative deficiency may therefore cause such a perinatal infection.

ACKNOWLEDGEMENTS

We are grateful for the advice and support given to us by Dr N. Datta and Professor J. H. Humphrey, both of the Royal Postgraduate Medical School, and Mr D. F. Hawkins of the Institute of Obstetrics and Gynaecology, Hammersmith Hospital. We also thank Dr M. Szelke of the Royal Postgraduate Medical School and Dr E. Hughes and her staff of the Neonatal Laboratory, Institute of Child Health for their help and advice and the use of their facilities.

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MATERNAL SERUM ALPHA-FETOPROTEIN SCREENING IN A PROVINCIAL HEALTH DISTRICT

BY

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Summary

Maternal serum alpha-fetoprotein (AFP) estimation as a routine screening test for neural tube defect (NTD) was introduced into the West Berkshire Health District, where 17 per cent of all deliveries and most of the antenatal care is undertaken by the general practitioners. In the first year, 4458 patients were screened and 43 of those (0·96 per cent) had raised serum AFP levels. Amniocentesis was performed on 31 patients (0·69 per cent). Ten fetuses with severe NTDs and one with exomphalos were detected and the pregnancies terminated. In six patients, raised serum AFP levels were due to fetal death. No normal pregnancy was terminated. Acceptability by patients was high. Provided that a good diagnostic ultrasound facility is available locally, maternal serum AFP estimation seemed to be a valuable screening test.

Brock *et al* (1973 *a* and *b*) were the first to suggest that maternal serum alpha-feroprotein (AFP) estimation might be useful as a screening test for gross fetal neural tube defect. Several other groups of workers agreed (Wald *et al*, 1973; UK Collaborative Study, 1977) and confirmed that the most accurate results were obtained between 16 and 18 weeks gestation.

In March 1977, routine serum AFP estimation was started to assess the practicability of screening all patients in the West Berkshire Health District, where 17 per cent of all deliveries were in the general practitioner maternity units and some patients were not referred to the consultant unit until the third trimester.

Serum was initially obtained at 14 to 20 weeks gestation, but this was narrowed to 16 to 18 weeks after publication of the UK Collaborative Study (1977). We now describe the introduction of the screening test and the first year's results.

METHODS

Organization

Circulars were sent to all general practitioners in the Health District advising them of the nature and purposes of the screening test. The Nursing Officer in charge of the general practitioner maternity units and the community services was asked to organize collection of samples through the practice and District midwives, as well as in the antenatal clinic.

Assay

Maternal serum AFP was measured in weekly batches by a radioimmunoassay method in the Radioisotope Laboratory at the Royal Berkshire Hospital, using standards supplied by the maternal serum AFP quality control scheme (BS 72/227).

For the first six months, results were expressed as centiles. After publication of the UK Collaborative Study (1977) results were given as multiples of the median value.

The antenatal clinic Sister was the one person informed of all AFP levels above the 95th centile or twice the median value. The Consultant involved was then told. All these patients had an ultrasound scan as a matter of urgency. If the raised AFP level was not attributable to wrong dates, multiple pregnancy or fetal death, and anencephaly was not diagnosed at ultrasound, a further maternal blood sample was obtained. Amniocentesis was done on those patients with repeat serum AFP levels greater than the 97th centile or 2·5 times the median value and only after full discussion with the patient and her husband.

Amniocentesis was done under ultrasound control and the amniotic fluid was analysed either by Dr N. Gregson at Salisbury, or in the Regional Genetics Unit at Oxford. With very few exceptions, a result was obtained within one week.

RESULTS

All patients screened in the first year of the study were delivered before the results were analysed. The hospital and general practitioner maternity unit delivery registers were scrutinized for any infants affected by NTD.

The screening test was introduced without major difficulties. The number of patients screened was 64·9 per cent at three months after introduction of the scheme, 80 per cent at seven months and over 85 per cent after that.

In the first year we screened 4458 patients (79·1 per cent of all bookings); serum AFP levels were reported as raised in 189 patients (4·2 per cent of those screened). After ultrasound scans and repeat serum AFP estimation there were 43 patients (0·96 per cent) with genuinely raised serum AFP levels (Table I).

All these patients agreed to further investigation and said they would request therapeutic abortion if the fetus was found to be abnormal. The outcome of these pregnancies is shown in Table II. Three patients aborted spontaneously before ultrasound, and in three others no fetal heartbeat was present. Four fetuses with anencephaly and two with spina bifida were diagnosed by ultrasound alone and the pregnancies terminated without preliminary amniocentesis.

Of the 31 patients (72 per cent) having

TABLE I

Analysis of patients reported as having raised serum AFP levels and in whom no further action was taken

Serum AFP level between 95th and 97th centile or 2 and 2·5 times the median value	97 patients
Wrong dates	44 patients
Twins not previously suspected	5 patients
	146 patients

TABLE II

Outcome of pregnancy in 43 patients with raised maternal serum AFP levels

	Number	Percentage of group with raised serum AFP levels
Spontaneous abortion before ultrasound	3	6·9
Fetus already dead at ultrasound	3	6·9
NTD diagnosed on ultrasound alone	6	13·9
Raised amniotic fluid AFP and NTD	4	9·3
Raised amniotic fluid AFP and exomphalos	1	2·3
Abortion after amniocentesis	1	2·3
Third trimester intrauterine fetal death	2	4·6
Hare lip	1	2·3
Normal live infants	22	51·2
	43	100

amniocentesis, five had amniotic fluid AFP levels diagnostic of fetal abnormality and therapeutic abortion was performed; four fetuses had spina bifida and one had an exomphalos. The remaining 26 patients had normal amniotic fluid AFP levels and 22 subsequently delivered normal live infants. There were two third trimester intrauterine deaths, one resulting from severe preeclampsia and intrauterine fetal growth retardation and the other unexplained. One patient was delivered of a live child with a hare lip.

One patient aborted a macerated fetus without apparent congenital malformation three days after amniocentesis. In this case, the ultrasound scan was restricted to placental location, and it is possible that the fetus was already dead at the time of amniocentesis.

No pregnancy with a normal fetus was terminated.

The screening programme was introduced in March 1977. The number of infants with an NTD born in the 12 months between July 1976 and June 1977 was 12. However, in the 12 months between July 1977 and June 1978 (when the impact of the screening programme could be felt) the number was only 3; and of these, 2 had closed defects.

There were two false negative results. One patient who had a normal serum AFP at 14 weeks delivered a baby with open spina bifida which died after four days. The second was in a patient who had had a previous infant with an NTD and a normal maternal serum AFP level at 15 weeks in the current gestation: anencephaly was diagnosed at a routine ultrasound scan and the pregnancy was terminated. Had the maternal serum AFP level been measured at 16 to 18 weeks the error might not have occurred.

In the year under review, in addition to the 31 amniocenteses already described, 41 second trimester amniocenteses were done for other reasons. Only one patient (mentioned above) aborted, giving an overall abortion rate of 1·3 per cent.

CONCLUSIONS

The introduction of maternal serum AFP estimation as a routine screening test has led to a reduction in the number of babies born with NTD from an expected 14 to 3, two of which were closed defects. One fetus with exomphalos was diagnosed and the pregnancy terminated. Although there were two false negative results, no normal pregnancy was terminated.

The cost of the screening programme was just under £1·00 per test including all materials and technician time. When set against the cost of special care for the severely affected infants this appears to be economically justifiable. Moreover, the psychological and emotional effects on the parents and siblings of an abnormal baby must be considered. The readiness with which all our patients with raised serum AFP levels accepted further investigation, and possible therapeutic abortion if the fetus was thought to be abnormal, confirms our belief that this is a worthwhile test. In addition, an increasing number of patients are now specifically requesting measurement of their serum AFP levels.

Implementation of the screening test was rapid and effective, much of the credit for which must go to the midwives and general practitioners. Although some patients are still not being screened, largely due to late booking with general practitioners, it is hoped that their number will decrease.

Some might raise ethical objections to the fact that only those patients with a raised serum AFP level were given details about the screening test and its implications. In future, we hope to include a note about serum AFP levels in our antenatal booklet so that those patients who object to such a screening test could ask for it not to be performed.

At least one good diagnostic ultrasound department at a central unit is an integral part of a screening programme such as we have described.

ACKNOWLEDGEMENTS

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ANTENATAL SCREENING IN OXFORD FOR FETAL NEURAL TUBE DEFECTS

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Summary

Between May 1975 and the end of 1977, 6443 antenatal patients were screened mainly between 16 and 22 weeks of pregnancy for neural tube defects (NTDs) at the John Radcliffe Hospital, Oxford, by maternal serum alpha-fetoprotein (AFP) measurement; a take-up of 72 per cent. Seventeen out of 18 (94 per cent) patients with open NTD pregnancies (9 out of 9 with anencephaly and 8 out of 9 with open spina bifida) had positive screening tests, and all except one were offered and accepted a termination of pregnancy. Two hundred and forty-five (3·8 per cent) patients with unaffected pregnancies also had positive screening tests, although only 1·4 per cent had an amniocentesis. Following ultrasonography, about 50 per cent of patients with unaffected pregnancies with positive screening tests were not offered an amniocentesis because they had a multiple pregnancy or their gestational age had been underestimated. The odds of having a fetus with an NTD among the women who had an amniocentesis was about 1 to 6 (1 to 11 for open spina bifida alone). Two apparently normal pregnancies were terminated. A survey of the acceptability of the screening programme among a consecutive sample of 73 patients who knew that they had a positive screening test revealed that all except one had no objection to screening in general, and 68 (93 per cent) wanted to be tested again in a future pregnancy. The approximate direct cost of the programme was £2 to £3 per patient screened, or about £1000 per NTD detected (about £2200 per open spina bifida detected).

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ANTENATAL screening for neural tube defects (NTDs) started in Oxford in May 1975, at first in only some of the antenatal clinics, but later in all, so that by 1976 all women who first attended one of the clinics at the John Radcliffe Hospital, Oxford, before 23 weeks of gestation were eligible for screening. This paper describes the experience of the screening programme until the end of 1977, the results of the programme, and its monitoring.

METHODS

DELIVERY OF THE SCREENING SERVICE

The alpha-fetoprotein (AFP) blood test

A special request card for the AFP test was routinely used. Patients who first attended the antenatal clinic after 22 weeks of gestation were not screened since it was felt that they would be too advanced in pregnancy for a termination to be offered if this was later found to be indicated. Within a few months of the start of screening, it became known that the screening test was relatively ineffective in the first trimester of pregnancy (Brock *et al*, 1975), and so women seen before 14 weeks of gestation were regarded as being too early in pregnancy for the test. By February 1977, 16 weeks was considered to be the earliest acceptable time (U.K. Collaborative Study on AFP in Relation to Neural-tube Defects, 1977). Patients who booked too early in pregnancy for the test were given the choice of returning to the Hospital Pathology Laboratory or visiting their family doctor for a blood sample to be taken after 16 weeks of gestation.

Clinicians were left to judge what to say to their patients about the screening test. In some cases a full explanation was given, but in other instances no specific mention of the test was made.

Communication of results

Positive results were reported to a single obstetrician with a special interest in antenatal diagnosis. A nurse with a special responsibility for the administration of the screening programme contacted the patient's family doctor and through him an appointment was made for an ultrasound scan. In some instances the family doctor raised the possibility of a fetal neural tube defect, but more often less specific information was given along the lines that gestational age was in doubt, and that there might be the possibility of twins.

Negative results were not reported individually but were available on special request. This avoided the considerable work and cost involved in the production and dispatch of large numbers of normal results, thereby enabling the screening staff to concentrate their efforts on patients needing special attention. To overcome the uncertainty that a sample sent to the laboratory might not have arrived safely, doctors who wished to have confirmation of safe receipt were asked to enclose a self-addressed envelope together with a short note of receipt, which was dated in the laboratory and returned. No patient is known to have had a sample sent to the laboratory without it having been tested.

Ultrasound scanning and amniocentesis

The scan was performed by radiographers at a special clinic held once a week. If the scan showed that a single fetus was present and the maternal serum AFP level was still elevated after any revisions of gestational age, the patient and her husband saw the obstetrician, who then discussed the possibility that the patient had a fetus with a neural tube defect. The potential benefits and hazards of amniocentesis were presented, and if the patient and her husband requested this, it was undertaken immediately.

Negative amniotic fluid AFP results were sent by post to the patient and her family doctor. If the result was positive the patient was normally seen by her own obstetrician, and if the patient chose to have a termination of pregnancy this was done under his care.

ANALYTICAL METHODS, NORMAL RANGES, AND CUT-OFF LEVELS

Maternal serum

Maternal serum AFP was measured using a single antibody radioimmunoassay. All samples were tested in duplicate and the mean used. At the start of screening in Oxford there were too few data to reliably estimate centiles greater than the 90th. It was known, however, that a cut-off level of 3·0 times the median value would approximately define a constant centile beyond the observed 97th centile at each week of gestation. Later, when over 1000 patients had had AFP tests, the 97th centile for singleton pregnancies for which the date of the last menstrual period was certain was estimated directly using a weighted logarithmic linear regression of the observed 97th centile against gestation, from 14 to 22 weeks of pregnancy. These figures were expressed as a multiple of the normal median, which was similarly estimated by regression. These were used as the screening cut-off levels, and at the end of 1977 were 2·5, 2·4, 2·3, 2·2, 2·1, 2·0, and 2·0 respectively for each completed week of gestation from 16 to 22 weeks of pregnancy. AFP results equal to or greater than these cut-off levels were considered positive and were, on average, reported five days after blood was taken.

Amniotic fluid

Amniotic fluid AFP was measured by 'rocket' immunoelectro-diffusion using 'Behringwerke' standards. All samples were assayed in duplicate in each of two separate analytical batches and the mean result used. An amniotic fluid AFP result was regarded as positive if it was more than 5 SD above the mean for the relevant week of gestation. It was usual to perform AFP tests on amniotic fluid samples collected for reasons other than suspected fetal NTD and in these circumstances, because the risk of NTD was low, a higher cut-off level (7 SD above the mean) was used if the *serum* AFP level was normal. The mean amniotic fluid AFP concentrations (and their SD) were calculated using a log linear regression of AFP on week of gestation from 15 to 22 weeks. The results were, in μg/ml respectively, 24·7 (6·59), 20·9 (6·20), 17·7 (5·83), 15·0 (5·48), 12·8 (5·16), 10·2 (4·85), 9·2 (4·56), 7·8 (4·29). After the start of the screening programme it was reported that quantitative measurement of cells which adhered rapidly to glass in the amniotic fluid cell cultures indicated the presence of NTDs (Gosden and Brock, 1977). While the significance of these cells remains uncertain their presence (rightly or wrongly) influenced management during part of our screening programme.

MONITORING THE AFP SCREENING SERVICE

When a patient attended the antenatal clinic for the first time in her pregnancy, a booking notification card was made out. The information on this card and the AFP request card were linked by computer, and routine statistics on the take-up and results of the AFP screening service were produced. Deliveries associated with the birth of an infant with an NTD were identified by examining the Delivery Suite birth register, the admissions register to the Special Care Baby Unit, and Pathology Department records. A delivery notification card was completed on all patients who were delivered at the John Radcliffe Hospital, and an attempt was made to link all women who booked at the John Radcliffe Hospital with all deliveries. This proved impractical on a routine basis because the follow-up of patients who did not complete their pregnancies or were not delivered in Oxford was too time-consuming and sometimes impossible.

To ensure that the proportions of NTD and non-NTD pregnancies above specified cut-off levels expressed in multiples of the median remained constant, the median AFP level and the cut-off levels were re-estimated after every 25 assay batches had been completed. In addition, the proportion of patients with AFP levels above the screening cut-off levels for each week was noted, and if over a period of two to three weeks this was greater than about 5 per cent or less than 1 per cent the assay was investigated. Regular control sera with AFP levels near the cut-off level were tested in each batch. If, over three to four assays, the results obtained from assaying these control sera were consistently higher or lower than their typical values the matter was investigated.

AD HOC ENQUIRY INTO ACCEPTABILITY OF SCREENING SERVICE

A survey was carried out on patients with positive serum AFP tests from the start of screening to the end of 1976 (the first 20 months). A questionnaire was sent by post to each of these patients, unless they had not been told that they had a positive result or if there was good reason to believe that such an enquiry might cause particular social or personal distress. If a reply was not received a second questionnaire was sent, and if this also was not returned the AFP screening nurse visited the patient.

Patients were asked who had informed them of their positive screening test (hospital doctor, general practitioner, nurse or midwife, medical secretary, or other person), and how this was done (in person, by telephone, or by letter). They were asked: if they were pleased that the test was done, even though the result may have worried them at the time; if they would like to be tested again in a subsequent pregnancy, and if so how they would like to be informed of the result should it be positive again, and if they had any criticism regarding screening for spina bifida in general, or regarding their own particular experience.

RESULTS

Overall survey—experience to the end of 1977

Table I shows that 6443 patients were screened from May 1975 to the end of December 1977,

TABLE I

Take-up of screening

	All pregnancies				NTD pregnancies			
	Year of booking				Year of booking 1975–77			
	1975	1976	1977	1975–77	Anencephaly	Open spina bifida	Closed spina bifida	All*
No. Booked	1199	3707	4079	8985	13	10	4	29
No. Screened								
at booking	652 (54%)	1609 (43%)	1343 (33%)	3604 (40%)	5	7	0	13 (45%)
after booking	175 (15%)	1012 (27%)	1652 (41%)	2839 (32%)	4	2	2	9 (31%)
at any time	827 (69%)	2621 (71%)	2995 (73%)	6443 (72%)	9	9	2	22 (76%)
No. not screened								
Booked too early and did not return	99 (8%)	153 (4%)	152 (4%)	404 (5%)	1	0	1	2 (7%)
Booked too late	186 (16%)	737 (20%)	711 (17%)	1634 (18%)	2	1	1	4 (14%)
Booked at an acceptable time, but not tested	87 (7%)	196 (5%)	221 (5%)	504 (6%)	1	0	0	1 (3%)
Total	372 (31%)	1086 (29%)	1084 (27%)	2542 (28%)	4	1	2	7 (24%)

* Includes two cases of fetal encephalocoele

TABLE II

Number of women with positive screening tests; number who had an amniocentesis, and number who had a termination of pregnancy

		NTD pregnancies			
	Non-NTD pregnancies	Anencephaly	Open spina bifida	Closed spina bifida	All* NTDs
No. screened	6421	9	9	2	22
No. with positive screening tests	245 (3·82%)	9	8	0	17 (77%)
No. who had an amniocentesis	91 (1·42%)	8	8	0	16 (73%)
No. of terminations of pregnancy	2 (0·03%)	9	7	0	16 (73%)

* Includes two cases of fetal encephalocoele

TABLE III

Details of open NTD pregnancies and amniotic fluid AFP false positives

		Maternal serum AFP test		Amniotic fluid AFP test				Termination or pregnancy
Pregnancy	No.	Gestational age (completed weeks)*	AFP (multiple of normal median)†	Gestational age (completed weeks)*	AFP (Standard deviations above mean)†	Blood-staining	Rapidly adhering cells (No. per 10 high-power field)	Gestational age (completed weeks)*
True positive								
Anencephaly	A1	17	7·4	20	42·9	Yes	ND[1]	21
	A2	18	8·6	19	34·3	Old Blood	ND	20
	A3	20	2·8	21	>41·8	Yes	ND	22
	A4	14	7·4	16	49·0	Pink	ND	17
	A5	15	9·6	18	61·1	Frank Blood	ND	19
	A6	21	3·1	No amniocentesis was performed				24
	A7	16	5·0	18	83·0	Yes	>600	19
	A8	17	3·9	18	87·6	Dark Brown	500	19
	A9	15	8·5	17	96·6	No	30	18
Open spina bifida	B1	16	4·1	19	11·0	No	ND	20
	B2	15	9·0	18	11·5	No	ND	20
	B3	16	4·5	17	14·1	No	ND	18
	B4	21 (15)	2·3 (5·2)	23 (17)	27·1 (16·1)	No	10	24 (18)
	B5	16	3·6	18	12·5	No	30	20
	B6	16	4·6	16	17·2	No	37	19
	B7	18 (14)	4·7 (9·0)	20 (16)	21·6 (15·3)	Yes	70	22 (18)
	B8	16 (13)	2·6 (4·2)	21 (18)	7·8 (5·5)	Yes	None	Not terminated
False positive								
NFAD	C1§	15	10·8	16	93·7	Yes	ND	17
,,	C2	— (17)	— (5·7)	— (17)	— (7·1)	No	None	— (19)
,,	C3	16 (15)	2·8 (3·3)	19 (18)	23·5 (21·7)	Frank Blood	46	Not terminated‡
IUD	C4**	17 (16)	3·5 (3·9)	18 (17)	62·4 (58·2)	Dirty Brown	<1	20 (19)
,,	C5	17 (14)	3·6 (5·3)	18 (15)	77·6 (63·0)	Dirty Brown	None	20 (17)
False negative								
Open spina bifida	D1	18 (13)	1·3 (4·0)	No amniocentesis was performed				Not terminated

* Gestational age was estimated using the date of the last menstrual period (LMP). Where this was not available or where the results of a scan significantly revised the gestational age, the age derived from the scan is shown in brackets.

† The AFP results were based on the gestational age using the LMP. Where the gestation was revised the revised AFP result is shown in brackets.

‡ A second amniocentesis one week after the first yielded clear amniotic fluid, with no rapidly-adherent cells and an AFP level of −0·02 SD above the mean.

§ Amniotic fluid albumin level was raised (6·0 mg/ml), consistent with a diagnosis of nephrotic syndrome; see Stirrat *et al*, 1979.

** Conjoined twins

ND = Not done.

NFAD = No fetal abnormality detected.

IUD = Intrauterine death.

representing 72 per cent of women who booked at clinics offering screening at the John Radcliffe Hospital. Forty per cent of patients had their AFP tests at the time of booking, and a further 32 per cent returned at a later stage of pregnancy for the test. About 11 per cent booked too early and did not return at a more suitable time in pregnancy, or booked at an acceptable time but were not screened, although at present the reasons for this are not known; a further 18 per cent booked too late. The modal week of booking was 15 weeks of gestation. Thereafter the number of patients who booked decreased until about 30 weeks and then remained steady (or even increased slightly) before declining to zero at term. Many patients who booked after 32 weeks received their routine care elsewhere, and in the event of screening being universally offered, would probably have been tested. The take-up of screening in those circumstances therefore can be expected to be greater than 72 per cent.

Table II shows the number and proportion of women with high AFP levels, the number who had an amniocentesis, and the number of terminations performed on account of an antenatal diagnosis of neural tube defect (NTD). Nearly 4 per cent of patients with unaffected pregnancies had positive screening tests (245/6421) and less than half of these subsequently had an amniocentesis—1·4 per cent of all the women screened. Ninety-four per cent (17 out of 18) of patients with an open fetal NTD (9 out of 9 with anencephaly and 8 out of 9 with open spina bifida) had positive screening tests. Therefore the odds of having an affected pregnancy was 1 to 14 given a positive screening test (1 to 31 for open spina bifida alone), and 1 to 6 given that an amniocentesis was undertaken (1 to 11 for open spina bifida alone).

Table III gives details of the 18 pregnancies with an open fetal NTD which were screened and the five unaffected ones with both positive maternal serum and amniotic fluid AFP tests. Twenty-one of these patients had positive amniotic fluid AFP tests (8 with fetal anencephaly, 8 with open fetal spina bifida, 2 with intrauterine fetal deaths, and 3 with unaffected fetuses) and 18 were offered a termination of pregnancy (9 with anencephaly, 7 with open spina bifida and 2 with unaffected fetuses (C1 and C2)) and all accepted. The patient (B8) with a fetal NTD and a positive amniotic fluid AFP test was not offered a termination because the AFP result was near the cut-off and there were no rapidly-adherent cells in the cultures. The other patient with a positive test who was not offered a termination of pregnancy had a second amniotic fluid AFP level which was normal. The two intrauterine fetal deaths probably occurred just before the amniocentesis, and one occurred in conjoined twins. One further patient (A6) with an anencephalic fetus had a positive screening test but did not have an amniocentesis since the diagnosis was made on the basis of the ultrasound findings alone. Further details relating to some of these patients (B8, C1, and C2) are presented elsewhere (Stirrat *et al*, 1979).

Of the 6443 patients who were screened, 6181 were regarded as having normal serum AFP levels (96 per cent). Of these, two had infants with closed spina bifida, and one had an open-fetal NTD (D1 in Table III). This last patient is discussed elsewhere (Stirrat *et al*, 1979).

In addition to the 6443 patients screened at the John Radcliffe Hospital, a further 667 patients who received their obstetric care from general practitioners were also screened. Among those patients one anencephalic fetus was detected and the pregnancy terminated. No NTDs were missed and no unaffected pregnancies terminated on account of screening.

TABLE IV

First 20 months of screening: number of women with positive serum AFP results, and number who were scanned in connection with screening

	Non-NTD pregnancies	NTD pregnancies
No. with positive screening test	131	9
No. negative after having a repeat test (no. repeat tests)	11 (21)	0 (0)
No. eligible for scanning	120	9
No. not scanned	28	0
No. scanned	92	9

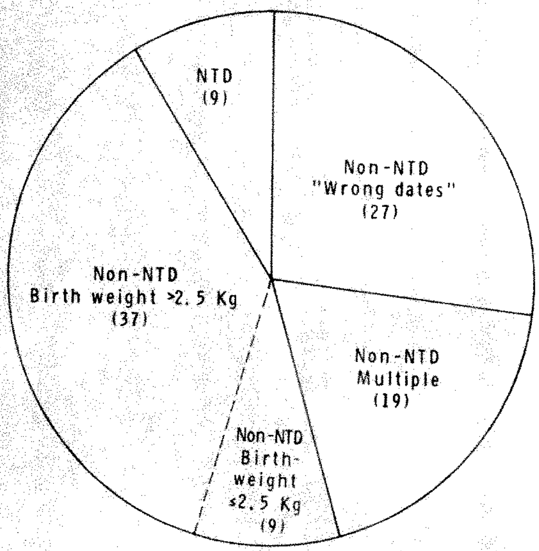

FIG. 1

The first twenty months of screening: the outcome of pregnancy among the 101 women with high serum AFP levels who were scanned in connection with screening. The numbers of women are shown in brackets. One pregnancy in the 'Non-NTD, birth weight <2·5 kg' group, and one pregnancy in the 'Non-NTD multiple' group ended in a spontaneous abortion.

Detailed survey—May 1975 to end of 1976

To investigate how patients with positive screening tests were selected for amniocentesis, all 140 patients in the category from the start of screening to the end of 1976 were followed up in detail (Table IV). There were 39 patients with high AFP levels who did not have an ultrasound scan, the clinician decided to take no further action in 28 patients mainly because they had AFP levels very near the cut-off level, the other 11 had normal AFP levels after a repeat test.

Figure 1 shows the outcome of pregnancy among the 101 women with positive AFP results who had an ultrasound scan in connection with screening, that is either had a scan because of the high AFP or were scanned earlier and the result used to re-interpret the AFP level. Nine were associated with NTD pregnancies. Among the 92 unaffected pregnancies scanned, 19 (21 per cent) were identified as being a multiple pregnancy, and among the remaining 73 unaffected singleton pregnancies, 27 (37 per cent) were associated with apparently high AFP levels due to the underestimation of gestational age. Eight (17·4 per cent) of the remaining 46 pregnancies were associated with infants born weighing 2·5 kg or less and one was associated with a spontaneous abortion. This high rate of low birth weight delivery has been described before (Wald *et al*, 1977).

Of those eligible for amniocentesis, three women (5·5 per cent) did not take up the offer, and seven (12·7 per cent) were not offered it because the clinician regarded the procedure as clinically contraindicated. One further patient (referred to in the overall survey) with an anencephalic fetus did not have an amniocentesis because the diagnosis was made using the scan result alone. Thus, from the original group of 140 patients with high serum AFP levels, 44 (31 per cent) subsequently had an amniocentesis.

The *ad hoc* enquiry into patient acceptability of the screening programme was done with 74 of the 80 patients who knew that they had a positive test. The 60 patients who did not know were not sent questionnaires. Of the six who knew but were not approached three were unmarried, one had a pregnancy complicated

TABLE V

Results of ad hoc *enquiry into patient acceptability of screening*

	No. patients	Per cent of replies
Questionnaires sent	74	—
Replies received	73	100
Method of informing patients of high serum AFP level (number satisfied with method)		
In person	51 (51)	70
By telephone	20 (9)	27
By letter	2 (1)	3
Pleased test was done	69	95
Not pleased	4	5
Would like to be tested in a future pregnancy	68	93
Would not like to be tested again	5	7
No general objection to screening	72	99
General objection	1	1
No objection to routine delivery of test	70	96
Wanted to choose whether to have test	3	4

by psychiatric problems unrelated to screening, one had a termination of pregnancy on psychiatric grounds, and one had a positive amniotic fluid AFP test which led to the termination of a normal fetus. Replies were received from all except one patient, who had emigrated. The results of the 73 questionnaires analysed are shown in Table V.

All 51 patients who had been informed personally that they had a positive screening test were satisfied by this approach, but 12 (55 per cent) of those informed by telephone or letter were not. Among patients informed personally, three said they would have preferred to have been informed by someone more knowledgeable about the screening service.

Four patients were not pleased that the AFP test was done. Three of these had religious or ethical objections to a test which might, after a series of further investigations, lead to a termination of pregnancy. The fourth patient did not disagree with antenatal diagnosis and selective abortion in principle, but she was generally dissatisfied with the antenatal care she had received, and felt that AFP screening had contributed to her dissatisfaction, since she had to wait three weeks for the result of her amniotic fluid AFP test, and was distressed during this time. The remaining 69 (95 per cent) were pleased that the test had been performed. Eight of the 44 patients having an amniocentesis felt that they had to wait too long for the results. As soon as this problem was identified it was corrected, and the patients were then notified of their results within five days if the test was negative. If it was positive the result was reported sooner (within 2 days) and the patient was directly informed as well as the obstetrician.

Only one patient objected to antenatal screening in general, although three felt that patients should have the opportunity to choose whether or not to have the test. The remaining 69 patients raised no objection to the test being performed routinely without a formal choice being offered.

Monitoring the screening service

The outcome of pregnancy was routinely determined by searching hospital records for births associated with a fetal NTD. It was assumed that pregnancies not identified in this way were unaffected. The reliability of this depends on the proportion of all births among screened patients which do not occur at the John Radcliffe Hospital and could not therefore be covered by our searches. In order to estimate this proportion, all 780 patients who had an AFP screening test in the first four months of 1976 were individually followed up and the outcome of pregnancy determined. Nineteen had a therapeutic, spontaneous, or missed abortion; a further 12 were delivered somewhere other than

TABLE VI

Monitoring print-out relating to patients tested in Assay Batches 176 to 200 from 28th September 1977 to 25th January 1978

	Completed Week of Pregnancy						
	16	17	18	19	20	21	22
AFP levels used in screening programme							
Median (ng/ml)	47·2	54·4	62·9	73·0	84·9	99·1	115·8
Cut-off (Multiple of median)	2·5	2·4	2·3	2·2	2·1	2·0	2·0
AFP levels observed in recent sample of patients tested							
Median (ng/ml)	48·0	52·5	60·9	67·2	83·2	114·5	150·5
10th centile (Multiple of observed median)	0·4	0·5	0·6	0·6	0·5	0·5	0·4
90th centile (,, ,, ,, ,,)	1·8	1·8	1·8	1·7	1·7	1·5	1·5
95th centile (,, ,, ,, ,,)	2·3	2·4	2·1	2·0	2·1	1·7	1·6
No. patients in recent sample							
Total	472	315	207	108	58	46	27
No. (%) with AFP levels equal to or greater than the cut-off level	15 (3·2%)	11 (3·5%)	6 (2·9%)	3 (2·8%)	3 (5·2%)	2 (4·3%)	2 (7·4%)

at the John Radcliffe Hospital, and the remaining 7 could not be traced. There was therefore a maximum of only 2·5 per cent of delivered patients on whom information regarding the outcome of pregnancy was not routinely available from the registers that were regularly examined, strongly suggesting that our case finding was practically complete. Indeed, only one patient with fetal NTD would be expected to be lost to follow-up every 17 years.

Table VI is an example of the information on a typical computer print-out relating to patients tested in a consecutive series of 25 assay batches. Such information is used to monitor the consistency of the median AFP level and the cut-off level over time. For the batches shown in Table VI, the medians did indeed remain stable, and 3·3 per cent of all patients were regarded as having high serum AFP levels.

DISCUSSION

The results of AFP screening in Oxford over the 32-month period from May 1975 to the end of December 1977, were consistent with those of the U.K. Collaborative Study of AFP in Relation to Neural Tube Defects, 1977, those of the Glasgow Screening Project (Ferguson-Smith *et al*, 1978), and those from Edinburgh (Brock *et al*, 1978). Eight out of nine pregnancies associated with open spina bifida and all nine patients with fetal anencephaly were detected (all 10 including the patient from general practice). About 4 per cent of patients were identified as having high serum AFP levels, and 1·4 per cent of patients with unaffected pregnancies underwent amniocentesis. The main reasons for not having offered an amniocentesis to patients with high AFP levels were the presence of multiple pregnancy, and the underestimation of gestational age revealed by ultrasound scanning. Among the 107 patients who had an amniocentesis on account of a high serum AFP level, the probability of having an NTD fetus was 1 in 6.

Some women with high serum AFP levels have already been scanned before blood is taken for their AFP screening test, and the results of such an ultrasound scan have sometimes been used to help interpret the AFP result, making it unnecessary for the patient to have another scan after the AFP result is known. In order to describe the sequence of events among the women with high serum AFP levels in our detailed 20 month survey, we considered any scan results that were used to help interpret the AFP results. All other scans were ignored. In this way scanning reduced the number of patients with singleton non-NTD pregnancies which required amniocentesis by just over one-third. In addition, it seems likely that the one patient with open fetal spina bifida in our study which was not detected by the AFP screening programme might have been detected had a scan been performed earlier (D1, Table III). The patient concerned was uncertain of her gestational age, and a policy of doing an ultrasound scan on all women with doubtful dates might be beneficial. It would not lead to a substantial increase in the number of women having this procedure, because a large proportion of women with doubtful dates are scanned anyway, albeit at a later stage of pregnancy. In Oxford, this policy would increase the number of women scanned by only 5 per cent.

It was not our practice to perform repeat serum AFP tests on patients, mainly because this would lead to delays of a week or more and subject patients to additional uncertainty. The advantages and disadvantages of repeat testing depend upon the details of the policy used. If a re-testing policy is used similar to the one suggested in the U.K. Collaborative Study on AFP in Relation to Neural Tube Defects (1977), this would reduce the number of unaffected pregnancies with high AFP levels by about one-third and also increase the detection rate to a small extent. The suggested policy was to re-test patients with AFP results within 20 per cent of a cut-off level of 2·5 times the median at 16 to 18 weeks of gestation, and the average of the two results taken to be positive if it was greater than the original cut-off level. Such a policy, would, however, mean an extra 5 per cent of patients would be recalled for second tests. If only patients with serum AFP values above a given cut-off level were re-tested, this would not only reduce the number of unaffected pregnancies with high AFP levels but it would also reduce the number of affected ones—that is, the detection rate would decrease. The policy regarding repeat testing has not been settled in Oxford and a decision will be made in the light of further findings. Although patients were not routinely re-tested some patients with high AFP levels, mainly those with AFP levels just above the cut-off level, did have repeat tests, and about half of these were subsequently considered to have normal results.

The question of consent to screening is a complicated one. While there is no disagreement that women who are offered an amniocentesis should be given a full explanation of the procedure, there is difference of opinion over the extent to which patients who have blood taken for a serum AFP screening test should be informed of the screening programme and its implications. The screening test itself is virtually without risk, but a few patients might like the opportunity to avoid the test, which might be regarded as unnecessary if they did not subsequently wish to have either an amniocentesis or a termination of pregnancy. This could be done only by giving *all* patients a full explanation of the screening programme which, for many women, might cause needless anxiety, since the test results would be normal in about 96 per cent of patients, and the fetus would be unaffected in about 99·5 per cent of cases. The question of consent was treated no differently from other clinical situations and was left to the discretion and judgement of the individual obstetrician.

The additional staff, reagents and equipment needed to undertake AFP screening in Oxford costs about £10 000 per annum. About one-third of this sum is devoted to research carried out in conjunction with the screening service. The approximate extra cost of the service, over and above those absorbed by existing services, is about £2 to £3 per patient screened, or about £1000 per NTD detected, or about £2200 per open spina bifida detected. We have not attempted to estimate the full economic cost of screening, by including such items as patient consultations, the collection of extra blood samples, and the staff and equipment needed to do the ultrasound scans and the amniocenteses. However, the practical economic questions which are likely to concern those considering the introduction of antenatal screening for NTDs are the extra costs rather than the total costs.

The screening service in Oxford has not only confirmed the results of the U.K. Collaborative Study (1977), but has also shown that such a service can be delivered effectively and compassionately by a small team of people without great additional cost. The results of the patient survey indicated that the service was, in general, welcomed and well received.

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MATERNAL SERUM ALPHA-FETOPROTEIN AND DIABETES MELLITUS

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Summary

Maternal serum alpha-fetoprotein (AFP) levels were measured from 12 to 24 weeks gestation in 27 singleton pregnancies in women with insulin-dependent diabetes mellitus and 90 controls without diabetes who were matched for gestational age. The geometric mean AFP level among the diabetic pregnancies was 60 per cent of that in the controls, a difference which was statistically significant ($P < 0{\cdot}01$). The difference in AFP level between diabetic patients and controls was most marked in sera taken before 21 weeks gestation.

WHILE undertaking an antenatal screening programme for neural tube defects by measuring maternal serum alpha-fetoprotein (AFP), it was noticed that one of the patients with unusually low levels had insulin-dependent diabetes mellitus (Bennett *et al*, 1978). Later it was noticed that two more diabetic patients had unusually low AFP levels at 17 weeks gestation, and this prompted us to undertake a matched study to investigate whether maternal serum AFP levels were indeed lower in diabetic patients than in unaffected patients.

METHODS

In Oxford a prospective survey of the outcome of pregnancy has been in progress for five years, and antenatal serum samples have been stored routinely at −40°C. From this survey 27 singleton pregnancies were identified in 24 patients with diabetes mellitus who required insulin treatment during their pregnancy. Details of the diabetic pregnancies are shown in Table I. The samples of serum collected in the course of routine antenatal care from 12 to 24 weeks gestation were retrieved from storage and 45 maternal serum samples were obtained from the 27 diabetic pregnancies. Each sample was matched with two others, one from each of two control singleton pregnancies without fetal neural tube defect, matching, to the day, for the gestational age at which the serum samples were obtained. They were also matched for the

TABLE I

Details of 27 pregnancies in patients with insulin-dependent diabetes mellitus from whom antenatal sera were collected between 12 and 24 weeks of gestation and assayed for AFP

Patient No.†	Age (years)	Duration of diabetes (years)	Parity: 28 weeks or more	+	Parity: Less than 28 weeks	Labour and delivery‡	Gestation at delivery (weeks + days)	State of infant	Sex of infant	Birth weight (g)	Birth weight centile*
1	22	16	0	+	2	Elective Caesar	38 + 6	Normal	M	2540	8
2	23	9	0	+	0	Spontaneous vaginal	36 + 4	RDS, hypoxia, hypoglycaemia	F	3190	74
3	26	3	2	+	0	Induced	37 + 6	Normal	F	3150	65
4a	32	11	2	+	0	Induced	39 + 1	Normal	M	3150	32
4b	34	14	3	+	3	Induced	38 + 5	Normal	F	2590	14
5a	26	4	0	+	0	Spontaneous vaginal	36 + 4	Normal	M	2980	52
5b	29	7	1	+	0	Elective Caesar	36 + 6	Single heart ventricle, NND	M	2660	30
6	24	22	0	+	1	Induced	37 + 6	Diabetic macrosomia	F	4200	>99
7	20	7	0	+	2	Elective Caesar	35 + 4	Normal	F	2340	26
8	22	10	0	+	0	Elective Caesar	37 + 0	RDS, polycythaemia	M	3800	94
9	28	20	1	+	0	Elective Caesar	39 + 5	Normal	F	3210	50
10	26	5	1	+	1	Elective Caesar	42 + 3	Normal	F	2320	<2
11	22	18	1	+	0	Emergency Caesar	42 + 2	Normal	F	2700	6
12	18	10	0	+	0	Intrauterine fetal death	36 + 2	—	M	2800	40
13a	22	11	0	+	0	Elective Caesar	37 + 2	Sacral agenesis	F	3870	97
13b	25	14	1	+	0	Emergency Caesar	34 + 6	Hypoglycaemia	F	3160	96
14	18	3	0	+	1	Elective Caesar	38 + 6	Normal	M	2640	11
15	37	6	3	+	0	Induced	38 + 2	Normal	M	3850	93
16	22	2	1	+	0	Induced	37 + 6	Normal	M	3630	88
17	23	<1	2	+	3	Induced	37 + 1	Normal	F	4370	>99
18	33	18	0	+	0	Elective Caesar	37 + 0	Normal	F	3530	87
19	25	9	2	+	0	Induced	37 + 2	Normal	M	3620	88
20	23	7	1	+	1	Elective Caesar	35 + 2	RDS hypoglycaemia	M	4020	>99
21	27	13	0	+	0	Induced	38 + 2	Normal	M	3100	40
22	22	13	0	+	0	Elective Caesar	35 + 0	RDS	M	2065	14
23	29	22	0	+	0	Emergency Caesar	37 + 6	Normal	F	4130	>98
24	20	<1	1	+	0	Elective Caesar	38 + 2	Hypoxia	M	3820	92

The mean birth weight was 3238 g (SD 640 g) and the mean gestational age at delivery was 37 weeks and 5 days (SD 1 week 6 days).

* Derived from Milner and Richards (1974).

† a and b refer to two pregnancies in the same patient.

‡ Delivery was vaginal except where otherwise specified; all the emergency Caesars followed spontaneous labour.

RDS = Respiratory distress syndrome.

NND = Neonatal death.

calendar quarter in which the samples were collected, so as to take account of any changes in AFP concentration which might occur with long-term storage. Gestational age was taken as

TABLE II

Maternal serum AFP levels from 12 to 24 weeks of gestation in patients with established diabetes mellitus, and in control patients

		Maternal serum AFP levels (ng/ml)				
Patient No.*	Gestation (weeks + days)	Diabetic patients (a)	Control		Geometric mean of controls (b)	Diabetic / Control mean (a/b)
			I	II		
1	12 + 6	2	28	9	16	0·16
	17 + 5	37	56	56	56	0·66
2	13 + 1	14	9	30	16	0·85
	21 + 1	77	130	211	166	0·46
3	17 + 0	8	36	119	65	0·13
4a	18 + 6	81	66	60	63	1·28
	21 + 6	112	216	140	174	0·64
4b	13 + 6	5	58	49	53	0·09
	16 + 6	6	112	66	86	0·07
5a	17 + 4	88	291	75	148	0·60
	23 + 4	168	164	112	136	1·23
5b	18 + 6	59	125	59	86	0·69
6	15 + 0	41	82	37	55	0·75
	23 + 1	145	145	72	102	1·42
7	13 + 0	31	5	25	11	2·80
	17 + 1	46	45	40	43	1·08
8	13 + 3	32	25	87	47	0·67
	19 + 6	100	108	96	102	0·99
9	13 + 2	4	41	19	28	0·16
	20 + 2	33	90	143	114	0·29
10	14 + 2	25	30	82	50	0·50
	18 + 5	97	57	86	70	1·38
11	13 + 5	22	66	26	42	0·54
	17 + 5	76	95	42	63	1·21
12	18 + 0	28	70	103	85	0·33
	21 + 4	154	78	164	114	1·35
13a	20 + 3	80	208	103	146	0·55
13b	12 + 2	16	18	12	14	1·09
	14 + 0	27	40	39	40	0·68
14	16 + 3	42	62	107	81	0·52
	20 + 2	83	98	141	118	0·71
15	21 + 2	95	126	104	115	0·83
16	12 + 2	10	15	15	15	0·70
	13 + 5	24	53	22	34	0·70
17	13 + 1	17	6	40	15	1·14
	21 + 1	92	54	89	69	1·33
18	12 + 2	14	4	21	9	1·47
19	14 + 6	16	101	49	70	0·23
20	24 + 4	235	287	80	151	1·55
21	13 + 0	55	16	45	27	2·05
	13 + 4	37	57	33	44	0·85
22	14 + 1	27	55	42	48	0·56
	17 + 1	38	66	53	59	0·65
23	19 + 1	129	128	56	85	1·52
24	18 + 3	45	129	123	126	0·36

* a and b refer to two pregnancies in the same patient.

the period of amenorrhoea from the first day of the last menstrual period as reported by the patient at her first antenatal clinic visit.

The antenatal sera were assayed for AFP in the same analytical batch using a single antibody radioimmunoassay, and the estimates were made without knowledge of details of the women or their pregnancies. Each sample was assayed twice and the geometric mean of the two results was used in the analyses.

RESULTS

The AFP levels in the diabetic and control pregnancies are shown in Table II and each value relating to a diabetic pregnancy is expressed as a multiple of the geometric mean AFP level for the two matched control pregnancies. Table III shows a summary of the data according to the gestational age at which the blood samples were taken. The geometric mean AFP level among the diabetic pregnancies was 60 per cent of that in the control pregnancies, if data relating only to the first serum sample tested are considered ($P < 0{\cdot}01$, Student's t-test using the log of a/b in Table II). If data from all the blood samples are considered, the mean level in the diabetic pregnancies was 65 per cent of that of the control pregnancies. The difference in AFP level between diabetic patients and controls was present in sera collected before the 21st week of pregnancy but not in those collected from 21 to 24 weeks.

TABLE III

Maternal serum AFP levels from 12 to 24 weeks gestation in patients with insulin-dependent diabetes mellitus and controls according to gestational age

		Geometric mean maternal serum AFP (ng/ml)		
Gestation (completed weeks)	No. of tests	Diabetic patients (a)	Controls (b)	Diabetic/control (a/b)
First test in each pregnancy				
12–14	15	15	25	0·59
15–17	4	34	81	0·42
18–20	6	63	95	0·67
21–24	2	150	132	1·14
12–24	27	27	46	0·60
All tests in each pregnancy				
12–14	18	17	27	0·64
15–17	9	32	68	0·47
18–20	10	66	96	0·69
21–24	8	127	124	1·03
12–24	45	37	57	0·65

DISCUSSION

Our results indicate that maternal serum AFP levels in the second trimester of pregnancy are lower in diabetic pregnancies than in unaffected pregnancies. The geometric mean corresponded to the 10th centile of normal for singleton pregnancies without neural tube defects routinely tested in Oxford.

The interpretation of whether or not an AFP level is low depends on a knowledge of the gestational age of the pregnancy, since in the second trimester of pregnancy serum AFP levels rise with increasing gestation. Since the fetuses of women with diabetes mellitus tend to be large in comparison with normal pregnancies, it is possible that obstetricians might tend to classify these patients as being more advanced in pregnancy than is in fact the case. Their AFP levels may then be regarded as low for their gestational age. This bias was avoided because the duration of pregnancy was based on the first day of the last menstrual period as reported by the patient.

It is possible, although unlikely, that the diabetic patients in our study inaccurately reported the date of their last menstrual period so that the duration of their pregnancies was overestimated. This would mean that diabetic patients would not only have low AFP levels for their gestational age before about 30 weeks, when AFP levels increase, but also have high levels after about 34 weeks when they decrease. To investigate this we tested the stored serum samples from diabetic patients who had blood taken and stored after 34 completed weeks of gestation. Seven of these patients were already in our study, and an additional one, who did not have blood collected at 12 to 24 weeks, was also tested. Nine serum samples from these eight patients were matched with control samples in the same way as before. The mean ratio of the AFP concentration in the diabetic pregnancies to that in the control pregnancies was 1·05, suggesting that the bias did not exist.

All the patients in the study were having insulin injections, and it is possible that the low AFP levels from 12 to 24 weeks of gestation were associated with the exogenous insulin rather than the diabetes itself. This, however, is unlikely because the low AFP levels were not evident later in pregnancy when, in general, the insulin dose given to patients was greater than that given earlier. We have considered a number of other explanations for our results. For example, the fetus of a diabetic mother might produce less AFP, or transfer less to the mother, than would be the case in normal pregnancies. However, we know of no evidence indicating that either explanation might actually apply. Therefore, while we regard our results as being real we cannot offer a plausible explanation.

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UNSTRESSED ANTEPARTUM CARDIOTOCOGRAPHY IN THE MANAGEMENT OF THE FETUS SUSPECTED OF GROWTH RETARDATION

BY

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Summary

A detailed analysis of the antenatal cardiotocographs (CTGs) in 57 patients with suspected fetal growth retardation is presented. Four traces were normal and 53 were 'non-reactive'. 'Non-reactive' traces can be sub-divided into three categories, which, in order of severity are, 'suspect', 'flat' and 'ominous'. When compared with the 'suspect' group, perinatal mortality was significantly increased and the Apgar scores at one and five minutes significantly decreased in the 'flat' and 'ominous' groups. The presence of fetal distress in labour was significantly higher in the 'flat' group as compared with the 'suspect' group. Intrauterine deaths occurred only in the 'ominous' group. With experience, ten patients later in the series with 'ominous' patterns were delivered within 24 hours of the detection of such and nine infants survived. At follow-up between 6 and 34 months after birth, psychomotor development was normal in 47 of the 49 surviving infants.

THE antepartum recognition of fetal growth retardation and its management continues to be a problem (Editorial, 1977). Using a 'non-stress' test in a group of 300 patients, we found a close correlation between heart rate and fetal activity patterns and the outcome for the fetus during labour and at delivery (Flynn and Kelly, 1977). We therefore applied this technique to patients showing poor intrauterine fetal growth, to ascertain if we could predict which fetus would die *in utero* and whether, by timely intervention, these babies would survive. We present our experience in this paper.

METHODS

The method of obtaining heart rate and fetal movements has been described (Flynn and Kelly, 1977). Between July 1975 and December 1977, 247 patients, with a clinical diagnosis of suspected fetal growth retardation had sequential cardiotocographs (CTGs) recorded during the antenatal period. Centile birth weights were calculated after adjustment for maternal height, weight and parity according to the tables of Thomson *et al* (1968) and fetal growth retardation was defined as a birth weight below the 10th centile (Thomson *et al*, 1968). At delivery, 57 babies were found to be growth retarded.

The clinical features are listed in Table I. In some patients the CTGs were started as early as 27 weeks. The number of traces obtained from any one patient varied from 4 to 50. Four CTGs were normal or reactive and 53 showed a non-reactive pattern, namely one or more of the following features was absent:

- (i) baseline fetal heart rate between 120 and 160 beats/minute;
- (ii) baseline variability between 5 and 20 beats/minute;
- (iii) accelerations of the fetal heart of 15 beats/minute to fetal movements and four such in the 20 minute period.

Because of the appearance of certain characteristics, these non-reactive tracings were further sub-divided into:

1. 'suspect' tracings showing normal basal fetal heart rate, baseline variability of 3 to 5 beats/minute and acceleration of the fetal heart with fetal movements;
2. 'flat' tracings showing normal basal fetal heart rate, baseline variability of 2 or less beats/minute and no acceleration of fetal heart with fetal movements;
3. 'ominous' tracings showing normal basal fetal heart rate, baseline variability of 2 or less beats/minute, no acceleration of the fetal heart with fetal movements and repetitive late decelerations.

TABLE I

Salient clinical features

Pre-eclampsia and chronic hypertension	17
Sickle cell anaemia	1
Diabetic pregnancy	4
Antepartum haemorrhage	3
Congenital abnormality of the uterus	1
Congenital abnormality of the baby	2
Intrauterine growth retardation without any other complication	29

TABLE II

CTG patterns in the growth-retarded fetuses

Pattern	Number of patients
Reactive	4
Non-reactive	53
Suspect	22
Flat	15
Ominous	16

A patient who at different times showed various patterns was ultimately classified by the worst pattern which in all cases was that one closest to delivery or intrauterine fetal death. The distribution of the various patterns is shown in Table II.

'Suspect' CTGs

When the suspect pattern occurred (53 patients) despite discontinuation of sedative drugs (Flynn and Kelly, 1977), the patient had CTGs on alternate days until she either went into spontaneous labour (20 patients), had an elective Caesarean section for an associated condition (2 patients) or the CTG became 'flat' (31 patients).

'Flat' CTG

When the CTG was 'flat', the patient was admitted to hospital. CTGs were obtained daily or more frequently until either an 'ominous' CTG was obtained (16 patients) or delivery was effected (15 patients).

'Ominous' CTG

Sixteen of the 'flat' CTGs went on to repetitive late decelerations and became 'ominous'.

Statistical evaluation was made using the χ^2 test with one degree of freedom.

RESULTS

There were 51 livebirths and 6 intrauterine fetal deaths; there were two early neonatal deaths. The relationship between perinatal mortality and the CTG patterns is shown in Table III.

Antepartum deaths

There were six antepartum deaths early in the study—all showed an 'ominous' pattern. Immediate delivery was not effected because of various factors, namely a clinical estimation of an extremely small baby and some uncertainty as to

TABLE III

Perinatal mortality

		Before labour	During labour	During the first week of life
Reactive		0	0	0
Non-reactive	Suspect	0	0	0
	Flat	0	0	1
	Ominous	6	0	1*
Total		6	0	2

* $(P < 0{\cdot}01)$

the exact period of gestation (four patients transferred to us for antenatal care after 26 weeks); suspected fetal abnormality (one patient); and patient refusal by one to accept medical advice. At that time the value of CTGs in the antenatal management of these patients had also not been fully established. Gestational age at delivery, however, was assessed to be between 32 and 36 weeks, the mean being 33·5 weeks; birth weight ranged from 1·0 kg to 1·6 kg with a mean of 1·27 kg.

Autopsy showed all fetuses to have died of asphyxia due to placental failure with no evidence of congenital anomalies. These patients had had daily CTGs over several weeks, or more frequently if the condition of the fetus appeared to deteriorate. Serial traces showed first a 'suspect' CTG, then a 'flat' CTG and finally an 'ominous' CTG; all six intrauterine deaths occurred within 12 to 24 hours of the appearance of repetitive late decelerations. Figure 1 shows late decelerations, bradycardia and then fetal death.

Neonatal deaths

There were two neonatal deaths. In one, the mother had fulminating pre-eclampsia (blood pressure 210/130 mm Hg and proteinuria of

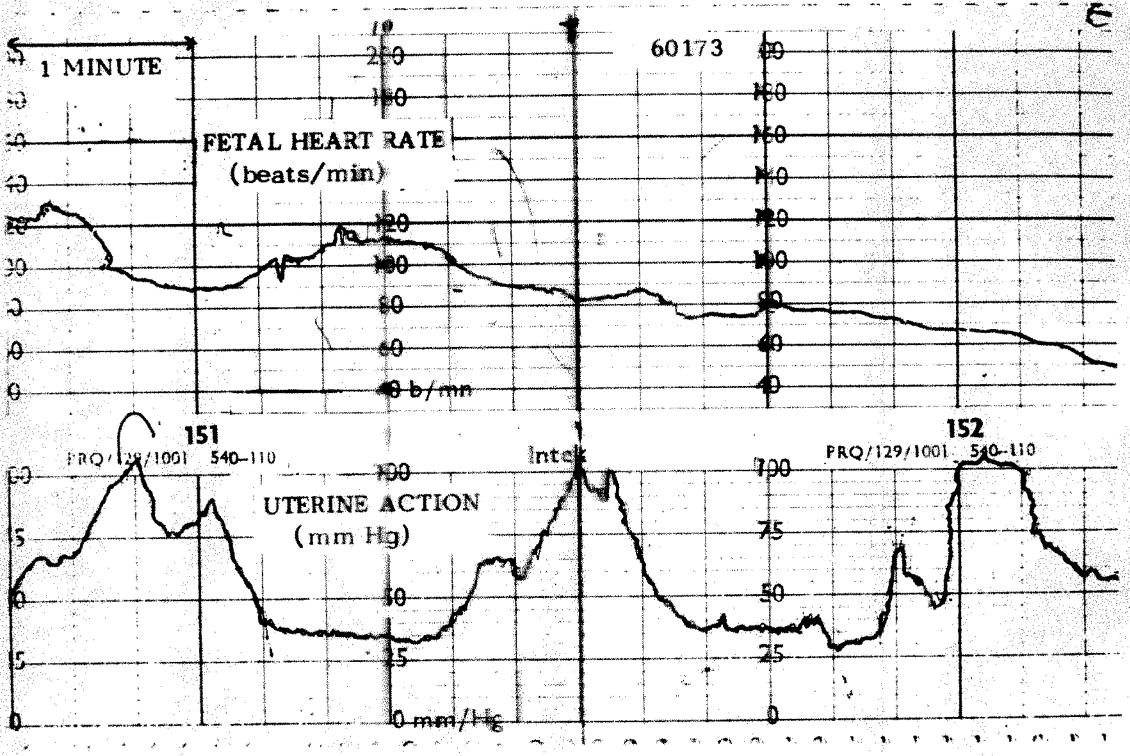

FIG. 1

Intrauterine fetal death: Repetitive late decelerations developing into a bradycardia and absence of fetal heart.

TABLE IV
Fetal distress

	Reactive	Non-reactive (n=53)		
		Suspect	Flat	Ominous
	(n=4)	(n=22)	(n=15)	(n=16)
Caesarean Section before Labour				
(a) For fetal distress	0	0	0	7*
(b) Other indications, e.g. diabetes, pre-eclampsia	1	2	3	0
Continuous monitoring in labour	3	20	12	3
Fetal distress in labour	0	4	10†	3
Caesarean section during labour for fetal distress	0	2	5	1
Operative vaginal delivery for fetal distress	0	1	3	1

* (P <0·01)
† (P <0·025)

6 g/day) superimposed on chronic hypertension. The trace was 'flat'. The patient was delivered by Caesarean section at 29 weeks. The male infant weighed 1·1 kg and died from prematurity at four days. In the other neonatal death, the mother had, at 36 weeks, an 'ominous' CTG. Spontaneous labour occurred that day and resulted in the delivery of an infant with trisomy 21 who died at the age of 23 hours.

Fetal distress

All patients other than those who had elective Caesarean sections (13 patients) and intrauterine deaths (6 patients) were continuously monitored during labour. The incidence of fetal distress and operative delivery are shown in Table IV. We diagnosed fetal distress during labour if the CTG showed late decelerations with or without loss of baseline variability and if fetal blood scalp samples showed a pH below 7·25.

Apgar scores

Table V shows the Apgar scores of 6 or less at one and five minutes. The Apgar scores for the patients with 'ominous' CTGs were significantly lower than those with 'suspect' CTGs.

Follow-up

The babies have been followed by our paediatric colleagues for 6 to 34 months. There were 43 infants who were followed for one year or longer, and 41 showed normal psychomotor development. One infant with trisomy 21 was developmentally retarded. One infant (birthweight 1·96 kg) whose mother had diabetes and epilepsy, had a 'flat' CTG and was delivered by elective Caesarean section at 37 weeks, had cerebral palsy and a cleft palate.

TABLE V
Apgar scores

	Reactive	Non-reactive		
		Suspect	Flat	Ominous
Apgar score 6 or less at 1 minute	0	3	6	11*
Apgar score 6 or less at 5 minutes	0	2	4	11*

* (P <0·001)

DISCUSSION

The dilemma of the clinician confronted with the management of the fetus suspected of growth retardation is to try and achieve the maximum maturity *in utero* without incurring the risk of an intrauterine death. Biochemical tests for fetal wellbeing such as urinary oestriol measurement or measurements of oxytocinase and human placental lactogen in blood, tend only to verify the clinical impression or indicate a worsening condition (Klopper, 1965; Spellacy *et al*, 1975; Edwards *et al*, 1976). CTGs have the advantage that the results are readily available

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and represent the immediate condition of the fetus. The 'unstressed' CTG, unlike the oxytocin challenge test, is easy to perform and may be repeated frequently without any danger to the fetus (Chew *et al*, 1977). However, excepting the paper by Visser and Huisjes (1977), which included but was not specific for the growth retarded fetus, no detailed study has been reported of the prognostic value of the unstressed CTG in the management of high risk pregnancies. We have attempted to do this by a more detailed classification of the non-reactive trace based on the amplitude of baseline variability and the presence of repetitive late decelerations. We were thus able to distinguish three groups of growth retarded fetuses giving different prognoses, 'suspect' with a good prognosis tending to go into spontaneous labour around 37 to 39 weeks and delivering babies not severely growth retarded, 'ominous' with a very poor prognosis unless there is urgent intervention and 'flat' an intermediate group in which the prognosis lay somewhere between the above two groups.

We found that the 'flat' CTGs continued for periods varying from three days to three weeks before becoming 'ominous' and that it was not possible to predict from the appearance of the 'flat' CTG the time of onset of the 'ominous' pattern.

It is our opinion, therefore, that while the appearance of a 'flat' trace is not an indication *per se* for immediate delivery, it is an indication for close observation and frequent CTGs in order to detect the onset of the 'ominous' pattern which requires urgent and decisive action. Our six intrauterine deaths occurred within 12 to 24 hours of the appearance of repetitive late decelerations. Following these deaths our present policy is to effect delivery within 12 hours of the appearance of the 'ominous' pattern. This requires the collaboration of a competent neonatal unit in order to avoid transferring antepartum deaths into the category of neonatal deaths or long-term infant morbidity. However, recent paediatric surveys (Stewart and Reynolds, 1974; Davies and Tizard, 1975; Stewart *et al*, 1977) show encouraging results and suggest that even in very small infants surviving the neonatal period, one may expect reasonable long-term development. This also has been our experience. We believe, therefore, that this classification offers to clinicians a possibility to achieve maximal intrauterine fetal maturity without prejudice to the growth retarded fetus.

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A STUDY OF BIRTH WEIGHT, PLACENTAL WEIGHT AND MORTALITY OF TWINS AS COMPARED TO SINGLETONS

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Summary

A study was made of 1655 sets of twins born between 1931 and 1975 in two clinical centres. Compared to singletons, twins were born three weeks earlier, weighed less at birth from 33 weeks to term and had smaller placentae from 21 to 22 weeks to term. The influence on birth weight of sex was smaller in twins, the influence of parity greater. Because, compared to singletons, lower placental indices were found in twins up to 37 to 38 weeks, the conclusion is drawn that the retardation of growth in twins is to some extent due to the placenta itself. When singletons and twins of the same gestational age were compared, the mortality was found to be similar, somewhat lower in twins up to 37 to 38 weeks and higher afterwards. Monochorial twins were found to be born earlier, weigh less at birth and have a higher mortality than dichorial twins. Placental weights were not different and the conclusion is drawn that the retardation of growth in monochorial twins is to some extent due to the higher incidence of marginal and velamentous insertions of the umbilical cord associated with lower birth weights.

COMPARED to singletons, twins are born earlier, weigh less at birth and have smaller placentae (McKeown and Record, 1952 and 1953*a*), but as in singletons (Lubchenco *et al*, 1963; Thomson *et al*, 1968) twin infants of multiparae are heavier than those of primiparae (McKeown and Record, 1953*b*) and boys are heavier than girls (Guttmacher and Kohl, 1958; Kloosterman, 1966; Thomson *et al*, 1968).

To understand the importance of the placenta in the intrauterine growth of twins, the placental index (placental weight divided by birth weight) in relation to menstrual age is of special interest (Van Bilderbeek, 1960; Gruenwald, 1970), but published findings disagree as to whether the placental index of twins is higher than (McKeown and Record, 1953*a*; Gruenwald, 1970), equal to (Ho and Wu, 1975) or lower than (Westermark, 1926) the placental index of singletons.

Monochorial twins are born earlier and weigh less at birth than dichorial twins (Van Bilderbeek, 1960; Naeye *et al*, 1966), possibly as a consequence of the method of placentation (Bulmer, 1970) rather than the size of the placenta.

In this cross-sectional study we compare data from a large number of twins with a standardized group of singletons. We will consider differences between birth weights and placental weights of singletons and twins and estimate the individual effects of placentation, parity and sex on gestational age at birth, birth weight, placental weight, placental index and perinatal mortality.

METHODS

The study is based on all 1655 twin pairs with a menstrual age over 16 weeks born at the Department of Obstetrics and Gynaecology of the Wilhelmina Gasthuis between 1931 and 1975 and at the Training School for Midwives in Amsterdam from 1948 to 1957. Forty-nine twin pairs of unknown gestational age (and a mortality of 20 per cent), 21 pairs with one fetus papyraceus and five pairs of conjoined twins were excluded from our study. Eighteen sets of twins in which one or both children died more than three days before birth were included only in the mortality statistics.

The gestational age of twins was compared with all 10 284 singletons of known maturity born after spontaneous onset of labour at the Wilhelmina Gasthuis in 1952, 1957, 1962, 1967, 1972 and 1975. A gestational age of n weeks was defined as $n \times 7 \pm 3$ days.

To compare the birth weights, placental weights, placental indices and mortality of twins and singletons, a group of singletons was chosen at random, standardized for year of birth, gestational age, sex and parity. A patient with a previous pregnancy of over 16 weeks was considered to be multiparous.

Infants were weighed almost immediately after birth. The placentation was decided from an examination of the membranes, and if necessary microscopic examinations were performed.

In 734 cases placental weights were recorded after all blood clots were removed, the membranes were trimmed off, the umbilical cord was cut within 2 cm and ligated after being allowed to bleed freely. The placenta was placed in a 4 per cent formalin solution and weighed within a week.

Statistical procedures used were mainly multiple regression analyses and analyses of variance. In many cases the calculations were performed using subgroups which differed in only one parameter, and the magnitude of resulting effects was calculated. All conclusions in the results which follow are statistically significant; for the sake of brevity we will not give the large number of F and p values.

RESULTS

As shown in Table I, 349 twin pairs (23·5 per cent) were monochorial and 1136 twin pairs (76·5 per cent) were dichorial, which is in agreement with Strong and Corney (1967).

Gestational age in singletons and twins

The mean gestational age in 10 284 singletons was 276·1 days (SD 21·0), 277·1 days (SD 19·7) for 5025 primiparae and 275·3 days (SD 22·2) for 5259 multiparae; the difference was not significant.

In 1562 twin pregnancies a mean gestational age of delivery was 255·7 days (SD 29·1), 20·4 days or almost three weeks less than in singletons. The frequency distribution of the gestational age in singletons and twins is given in Figure 1. The modal value for twins was 39 weeks or only one week earlier than for singletons, but 13 per cent of singletons and 51 per cent of twins were born before 38 weeks. Monochorial twins were found to have a significantly shorter duration of pregnancy than

TABLE I

Placentation, parity and sex of 1485 twin pairs

		Sex	No. of twin pairs
Monochorial twins (n = 349)	Primiparae	MM	81
		FF	73
	Multiparae	MM	94
		FF	101
Dichorial twins (n = 1136)	Primiparae	MM	151
		FF	133
		MF	154
	Multiparae	MM	201
		FF	189
		MF	308

M = male. F = female

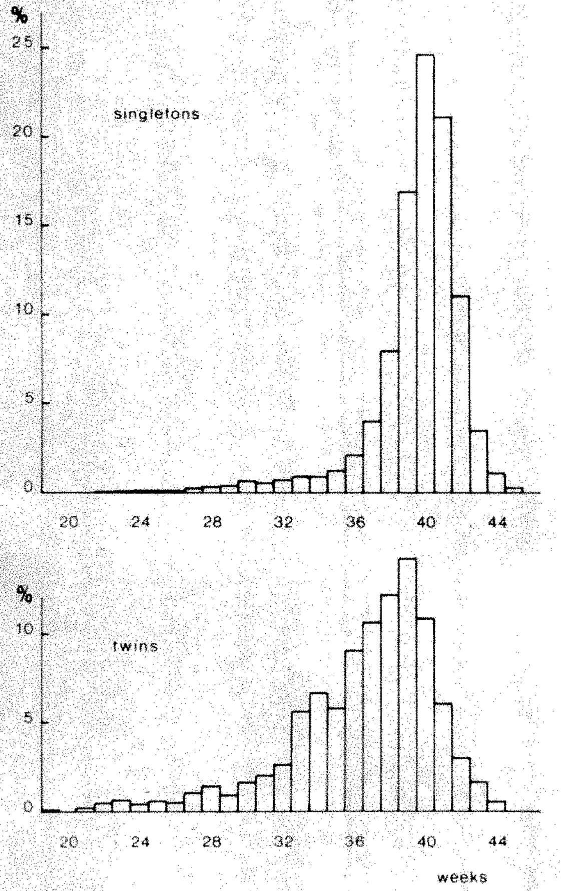

FIG. 1

Frequency distribution of the gestational age in singletons and twins.

dichorial twins by 10·6 days. The mean gestational age of twin pregnancies in multiparae was found to be 3·9 days longer than in primiparae; the difference was stastistically significant. Fetal sex had no effect.

Birth weights in singletons and twins

The mean birth weights of singletons and twins in relation to gestational age have been studied previously (Bleker *et al*, 1977*a*). Compared to singletons, the mean birth weight of twins was smaller from about the 33rd week, the difference at 39 to 40 weeks being about 600 g. In this study we found the newborn infants of multiparae to be heavier than those of primiparae in both singletons and twins. The mean effect of parity was 132 g (SE 17) in singletons and 152 g (SE 15) in twins.

Boys were heavier than girls in both singletons and twins. The mean effect of sex was 121 g (SE 16) in singletons and 105 g (SE 15) in twins. Dichorial twin infants were found to be heavier than monochorial twin infants, the mean difference was 95 g (SE 18).

Polyhydramnios

We considered the incidence of the clinical diagnosis of polyhydramnios in twins in relation to placentation and the duration of pregnancy from 691 twin pairs born between 1958 and 1977. Thirty of 205 monochorial twin pairs (14·6 per cent) and 10 of 486 dichorial twin pairs (2·1 per cent) were found to be associated with hydramnios. The mean gestational age of all monochorial twin pairs was 242·1 days, but when cases associated with hydramnios were excluded the mean gestational age was 250·9 days.

Placental weight of singletons and twin infants

The mean placental weights of 1468 singletons and 734 twins in relation to gestational age are shown in Figure 2. As compared to singletons the mean placental weight of twins found was to

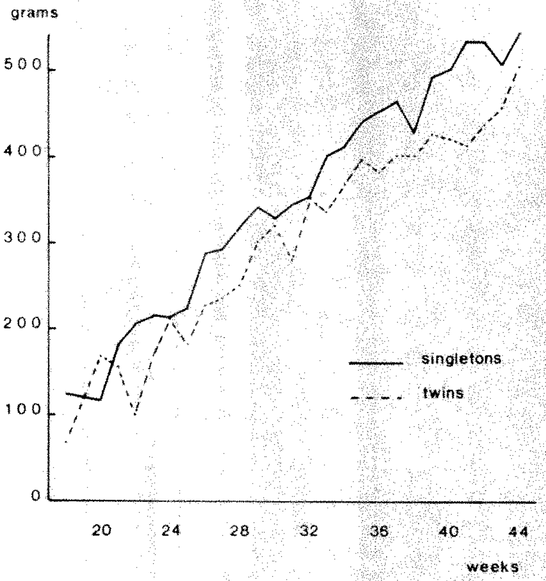

FIG. 2

Mean placental weights for singletons and twins in relation to gestational age.

be consistently smaller from 22 weeks to term, the mean difference at 39 to 40 weeks being 70 g. Placentae of multiparae were found to be heavier than placentae of primiparae in both singletons and twins. The mean effect of parity was 21 g (SE 5 g) in singletons and 17 g (SE 4 g) in twins. No effect of sex was found in either singletons or twins, and mean placental weights were the same in both monochorial and dichorial twins.

Placental index of singleton and twin infants

The mean placental indices of 1460 singletons and 730 twins in relation to gestational age are given in Figure 3. Compared to singletons, we found a lower placental index in twins from 21 to 22 weeks up to 37 to 38 weeks, after which twins had a higher placental index. Parity had no significant effect on the placental index in either singletons or twins. Since males are heavier at birth than females whereas the placental weights are the same in both sexes, one would expect the placental index to be lower in males; this was true in the case of twins but not in singletons. Monochorial twins were found to have higher placental indices than dichorial twins.

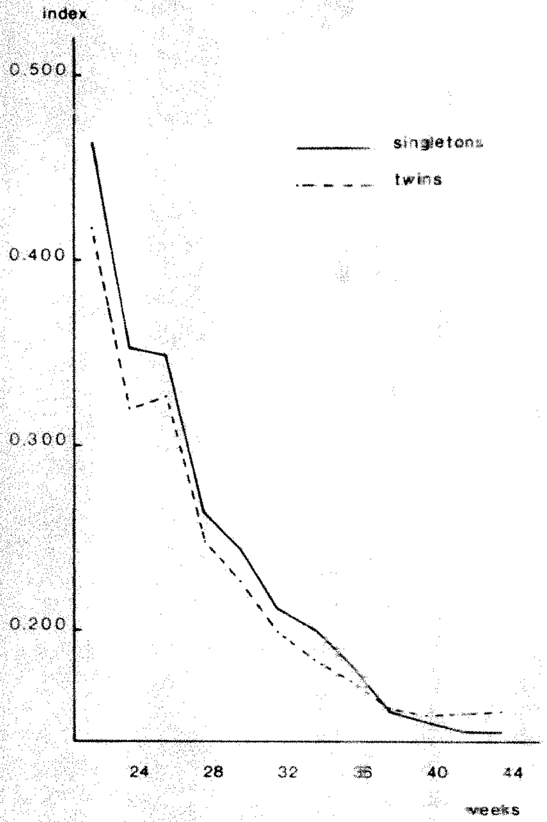

FIG. 3
Mean placental indices of singletons and twins in relation to gestational age.

Mortality in singletons and twins

The perinatal mortality of singletons and twins in relation to gestational age is given in Figure 4

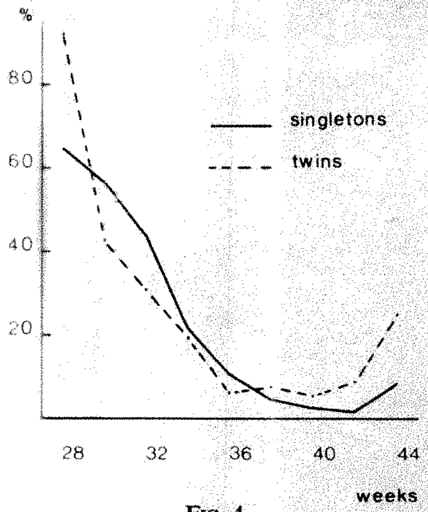

FIG. 4
Perinatal mortality of singletons and twins from primiparae in relation to gestational age.

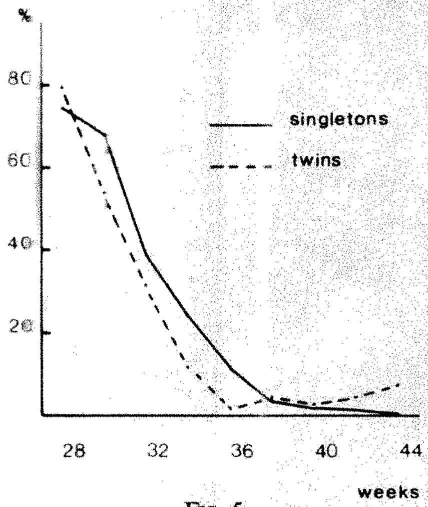

FIG. 5
Perinatal mortality of singletons and twins from multiparae in relation to gestational age.

(primiparae) and Figure 5 (multiparae). In agreement with Kloosterman (1956), mortality in singletons was found to decrease continuously in multiparae and to increase after 41 to 42 weeks in primiparae. Mortality in twins was found to be lower than mortality in singletons until 37 to 38 weeks, after which mortality in twins exceeded that in singletons, particularly after 39 to 40 weeks and in primiparae. The overall mortality after 16 weeks gestation of all singletons and twins is given in Table II; no important differences were found because the high mortality in twins is largely due to the high incidence of prematurity (Potter, 1963), and the singleton controls in this study were matched for gestational age. The mortality of second born twins was found to be higher than that of firstborns, to a large extent due to a higher mortality in the first week of life (Table II). The perinatal mortality (pregnancies of 28 weeks or more) of singletons and twins, according to placentation, parity and sex is given in Table III. The perinatal mortality of monochorial twins was found to be almost twice as high as of dichorial twins; even at the same gestational age the perinatal mortality was found to be higher in monochorial twins. Except in monochorial twins of primiparae, perinatal mortality was

TABLE II

Mortality in singletons and twins with a gestational age over 16 weeks

	No. of children	Antepartum mortality (per cent)	Intrapartum mortality (per cent)	First week mortality (per cent)	Total mortality (per cent)
Singletons	3160	2·3	4·6	7·7	14·6
Twins	3160	2·8	2·7	8·4	14·0
First born	1580	2·4	2·8	6·8	12·1
Second born	1580	3·2	2·6	10·0	15·8

Singletons and twins were matched for period of gestation, the effect on mortality due to pre-term labour is therefore the same in both groups.

TABLE III

Perinatal mortality in singletons and twins in relation to placentation, parity and sex (pregnancies of 28 weeks or more)

	Parity	Sex	No. of children	Antepartum mortality (per cent)	Intrapartum mortality (per cent)	First week mortality (per cent)	Perinatal mortality (per cent)
Singletons	Primiparae	M	629	2·2	4·1	6·8	13·1
		F	568	1·7	4·6	5·3	11·6
	Multiparae	M	924	1·4	4·5	6·5	12·4
		F	907	1·5	3·0	3·4	7·9
	All		3028	1·7	4·0	5·4	11·1
Monochorial twins	Primiparae	M	153	6·5	5·2	8·5	20·2
		F	135	5·9	4·4	11·1	21·4
	Multiparae	M	176	1·1	2·3	7·9	11·3
		F	184	1·6	1·1	6·5	9·2
	All		648	3·6	3·1	8·3	15·0
Dichorial twins	Primiparae	M	446	2·2	2·5	6·3	11·0
		F	410	2·0	2·0	5·2	9·2
	Multiparae	M	702	1·3	1·3	5·0	7·6
		F	678	1·8	0·9	3·8	6·5
	All		2236	1·8	1·6	5·0	8·4

Singletons and twins were matched for period of gestation, the effect on mortality due to pre-term labour is therefore the same in both groups.

found to be higher in boys than in girls, and higher in primiparae than in multiparae in all groups (Table III).

DISCUSSION

The mean gestational age of twins was found to be 20·4 days shorter than that of singletons. McKeown and Record (1952), who only considered pregnancies of 28 weeks or more, found a difference of 18·9 days, and for pregnancies of 28 weeks or more in our data, we found a difference of 18·1 days, which is in good agreement. In common with Van Bilderbeek (1960) and Gruenwald (1970), we found a shorter duration of pregnancy in monochorial twins than in dichorial twins; this difference might be due to the extra distension of the uterus caused by polyhydramnios, which occurs more often in monochorial twins (Van Bilderbeek, 1960) and is known to shorten the duration of pregnancy (Van der Velden, 1957). McKeown and Record (1952) found higher birth weights in multiparae than in primiparae, and because the duration of pregnancy was similar, the conclusion was drawn that the amount of distension which the uterus will tolerate at a given period of pregnancy is greater in multiparae than in primiparae. We found a significantly longer duration of twin pregnancies in multiparae and therefore support their conclusion.

Intrauterine growth is dependent not only on the duration of pregnancy but also on factors which include sex, parity, and placental factors (Kloosterman and Huidekoper, 1954; Kloosterman, 1966; Thomson *et al*, 1968). We found male infants to be heavier than females in both singletons and twins, the mean effect of sex being of less importance in twins. Possibly due to our smaller numbers, we could not confirm the lower placental indices which Kloosterman and Huidekoper (1954) found in singleton males as compared to females. In twins, however, we could confirm their findings. Lower placental indices in males means relatively less placental mass available to the male fetus and might to some extent be responsible for the higher perinatal mortality we found in males (Table III).

For parity, we found birth weights and placental weights to be higher in multiparae than in primiparae in both singletons and twins, the mean effect due to parity being of more importance in twins. Because we found less influence of sex and more influence of parity on birth weight in twins than in singletons we agree with Naeye (1964) who suggested that environmental factors limit fetal growth in twins. Since the placental indices of multiparae and primiparae were the same, we may conclude that multiparae, possibly by an altered uterine vascular system developed in the previous pregnancy, offer a more favourable environment for the development of the placenta associated with better fetal development. Moreover the lower perinatal mortality and stillbirth rate in multiparae suggests that they have a greater reserve capacity than primiparae.

The birth weight of twin infants is lower than of singletons from 33 weeks to term (Bleker *et al*, 1977*a*), but the mean placental weight available to each twin was found to be lower than for singletons from 22 weeks to term (Fig. 2). We agree with Van Bilderbeek (1960) that twins have a lower placental index up to 37 to 38 weeks (Fig. 3) and that the placenta may limit growth. On the other hand, McKeown and Record (1953*a*) and Gruenwald (1970) who found higher placental indices in twins concluded that relatively more placental tissue was available to the fetus and therefore the retardation of growth in twins must be due to influences outside of the placenta.

At longer gestational age heavier placentas were recorded (Fig. 2), but according to Kloosterman (1965) that observation should not lead to the conclusion that the placenta is growing until the end of pregnancy rather that pregnancies associated with large placentas last longer. In a previous longitudinal

TABLE IV

Insertion of the umbilical cord in 1002 placentae of twin infants

Insertion of the umbilical cord	Monochorial twins		Dichorial twins	
	Number	Per cent	Number	Per cent
Central	24	8·0	132	18·7
Paracentral	50	16·8	188	26·7
Lateral	126	42·3	285	40·5
Marginal	66	22·1	75	10·6
Velamentous	32	10·7	24	3·4

TABLE V

Birth weights in relation to the place of insertion of the umbilical cord in singletons and twins

Insertion of the cord	Singletons (39 to 41 weeks)*		Monochorial twins (37 to 40 weeks)		Dichorial twins (37 to 40 weeks)	
	No. of infants	Mean birth-weight (g)	No. of infants	Mean birth-weight (g)	No. of infants	Mean birth-weight (g)
Central	206	3428	11	2829	64	2639
Paracentral	444	3471	20	2671	98	2708
Lateral	1696	3456	51	2613	120	2668
Marginal	236	3375	21	2502	32	2537
Velamentous	38	3131	9	2384	10	2377

* Kloosterman, 1966.

study we could confirm that the human placenta does not grow until the end of pregnancy but stops growing several weeks earlier (Bleker *et al*, 1977*b*), so that the relatively higher placental indices we found in twins after 37 to 38 weeks might indicate that these pregnancies lasted longer because of their large placentae. Even so these large placentae are not able to prevent the increasing mortality of twins after 37 to 38 weeks, possibly due in part to lack of maternal reserve capacity and also because of problems associated with twin delivery unassociated with placental reserve.

Apart from placental size, the method of placentation might be of importance in the intrauterine growth of twins (Bulmer, 1970). In agreement with Gruenwald (1970), monochorial twins were found to have lower birth weights than dichorial twins at all gestational ages. Placental weights were found to be the same and so placental indices were higher in monochorial twins. Thus monochorial twins use their placentae less effectively, possibly due to their method of placentation. Because Adair and Thelander (1925) and Kloosterman (1966), unlike Uyanwah-Akpom and Fox (1977), found an infavourable influence of a marginal or velamentous insertion of the umbilical cord on birth weight of singletons, we studied the incidence of these conditions in twins. As shown in Table IV we found a marginal or velamentous insertion of the cord more than twice as often in monochorial twins than dichorial twins, and a strong suggestion (Table V) that such insertions of the cord have an unfortunate effect on birth weights in twins. We suggest that monochorial twins weigh less than dichorial twins because they make less effective use of their placentae, due to the higher incidence of abnormal vascular architecture.

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NOTE

More detailed tables and information can be obtained from the authors.

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THE COMPARTMENTAL DISTRIBUTION OF OESTROGENS AND PREGNANCY SPECIFIC β_1 GLYCOPROTEIN

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Summary

Pregnancy specific β_1 glycoprotein (PSβ_1G), unconjugated oestriol, total oestriol and oestradiol were measured in the peripheral venous blood of 25 pregnant women. The results were compared with similar measurements in maternal tissue fluid, cerebrospinal fluid, and uterine vein blood; or with retroplacental blood, umbilical cord blood, and amniotic fluid. The highest mean concentration of PSβ_1G occurred in maternal venous blood and was 17·37 (SD 7·07) mg/dl. The mean peritoneal value was 21 per cent of the mean peripheral vein value. Two tissue fluid values were 32 per cent and 5 per cent of the corresponding peripheral vein values. No measurable amount of PSβ_1G was found in maternal cerebrospinal fluid and cord artery. Unconjugated oestriol was found in highest concentration in retroplacental blood. The concentration fell from here to contiguous compartments. An exchange between these compartments was suggested by significant correlations in their oestriol content. Conjugated forms of oestriol, as represented by the measurement of total oestriol, were present in highest concentration on the fetal side in cord arterial blood, but could be found in all other compartments. Oestradiol appeared in highest concentrations in retroplacental blood. Low values of oestradiol were found in the fetal compartment. Correlations between maternal compartments were few and this was considered evidence of impeded transmission between these compartments.

THIS study was designed to examine the spread of oestrogens through the fetal and maternal compartments from their point of production in the fetoplacental unit, and to compare this with the distribution of pregnancy specific β_1 glycoprotein (PSβ_1G). Although the final step in the biosynthesis of oestriol and oestradiol probably takes place in the placenta, it seemed relevant to examine separately the distribution of the fetoplacental oestrogen, oestriol, and of the purely placental oestrogen, oestradiol. The molecular sizes of oestriol and its various conjugates such as oestriol-3-sulphate and oestriol-16 glucosiduronate are not greatly different, but their lipophilic characteristics and hence their likely transmission rates across the membrane barriers between compartments differ greatly. We have therefore measured separately unconjugated oestriol and total oestriol, a mixed entity consisting almost entirely of the conjugated forms. For contrast we have examined the distribution of a much larger molecule, the protein PSβ_1G which, like the steroids, is also believed to originate from the trophoblast (Tatarinov *et al*, 1976), expecting that, because of its size, the pattern of its distribution should be quite different. It was hoped that this comparison of the compartmental distribution of various placental products would give some insight into the site and nature of their physiological action in pregnancy.

SUBJECTS AND METHODS

Twenty-five patients consented to the collection of samples. These were mainly women having an elective Caesarean section within a week of term. Permission for the study was obtained from our local ethical committee.

Two samples of tissue fluid were collected in different ways. In one case on the day before delivery a small intravenous cannula was placed in the pretibial pad of oedema. The small sample obtained was just sufficient to measure PSβ_1G and relate this to a simultaneous venous blood value. A second sample of tissue fluid from another patient was obtained from an excised abdominal scar with its underlying fat, which exuded sufficient fluid in the time taken to complete the operation for all the parameters to be measured.

Cerebrospinal fluid was collected at the time of spinal anaesthesia. Peripheral venous blood was taken before delivery of the baby. Only clear samples of peritoneal fluid and amniotic fluid were analysed. Uterine vein blood was withdrawn from one of the wide bore veins running lateral to the uterus in the broad ligament; any bleeding which followed ceased as soon as the baby was delivered. Cord artery and vein blood was collected after clamping and dividing the cord and before delivery of the placenta. Retroplacental blood, taken to be the dark blood which appears from behind the separating placenta, was allowed to run into a small bowl at the uterine incision. The heparinized blood samples were separated after centrifugation and the plasma stored at −20°C. Samples from the same patient were measured on the same assay run. Unconjugated oestradiol was measured by a specific radioimmunoassay, using antisera

TABLE I

Oestrogens and PSβ_1G in maternal and fetal compartments

Placental product		Interstitial fluid	Cerebrospinal fluid	Peritoneal fluid	Peripheral vein	Uterine vein	Retroplacental blood	Cord vein	Cord artery	Amniotic fluid
PSβ_1G	Range	1·34, 4·8	<0·2	1·14–6·37	5·62–32·80	5·83–33·62	2·84–25·62	<0·2–0·44	—	<0·2–0·59
(mg/dl)	Mean	3·07	—	3·71	17·37	16·55	12·63	0·07	<0·2	0·19
	SD	—	—	1·88	7·07	7·39	6·54	—	—	—
	No.	2	2	12	23	17	20	16	7	9
Total	Range	—	30, 38	256–1223	417–1584	330–1965	800–5356	3337–9295	5460–14370	1057–6336
oestriol	Mean	717	34	773·1	879·1	993·6	3229·2	6572·7	7774·2·	4045·8
(nmol/l)	SD	—	—	364·9	350·0	426·4	1294·5	1572	3003·8	1772·2
	No.	1	2	13	22	18	20	16	7	5
Unconjugated	Range	—	6, 17	17–48	18–109	29–285	306–2576	125–449	48–176	24–166
oestriol	Mean	70	11·5	38·1	57·1	107·1	840·5	260·1	100·4	75·83
	SD	—	—	15·2	26·3	74·6	557·9	99·0	38·1	48·33
(nmol/l)	No.	1	2	13	22	19	20	16	7	6
Oestradiol	Range	—	—	13–79	33–156	53–385	54–510	12–67	5–21	2·2–5·0
	Mean	4·1	0·53	46·5	98·9	163·1	245·4	35·7	14·3	3·45
(nmol/l)	SD	—	—	19·54	37·6	99·6	140·4	14·4	5·5	0·93
	No.	1	1	10	21	19	19	16	9	5

raised against oestradiol-6-(O-carboxymethyl) oxime-bovine serum albumin. Unconjugated oestriol was extracted by ether, and total oestriol was measured after ether extraction of fluid previously hydrolyzed by acid. Oestriol was measured in the ether extracts according to the method previously described by Wilson (1975). PSβ_1G was measured by a modified Laurel rocket technique as described by Bruce and Klopper (1978).

Results are presented as range, mean and standard deviations. As the parameters do not have a normal distribution, Spearmans rank order correlation ρ has been used to relate values in one compartment with another. To establish the pattern of their distribution between the compartments, values in one compartment were expressed as a percentage of the peripheral vein value and the mean calculated.

RESULTS

Table I shows the mean values found in the various compartments. Figure 1 shows the comparative distribution of each parameter, excluding tissue fluid and cerebrospinal fluid. The two values were 5 per cent and 32 per cent of the maternal vein values in the case of PSβ_1G, 94 per cent in the case of unconjugated oestriol, 43 per cent for total oestriol and 5 per cent for oestradiol. PSβ_1G was not detectable in cerebrospinal fluid by our technique but unconjugated oestriol was 21 per cent and 57 per cent of the peripheral vein values, total oestriol 6·8 per cent and 2·4 per cent. In only one patient was there sufficient material for an oestradiol estimation on cerebrospinal fluid which was 0·7 per cent of the peripheral vein value.

PSβ_1G

The crude comparison of mean values shows a diminution of PSβ_1G from peripheral blood to uterine vein, retroplacental blood and peritoneal fluid. Useful comparisons can be made in certain of these compartments. Uterine vein values were less than peripheral vein values in 4 patients, there was no difference in 8, and in 5 patients the uterine vein value was greater. Statistical analysis showed no significant difference. Retroplacental blood values were less than their respective peripheral vein values in 16 patients, no different in one, and greater in three; the overall difference was statistically significant ($p < 0{\cdot}05$). Peritoneal fluid values were 21 per cent of peripheral vein values. No measurable amounts of PSβ_1G were found in cerebrospinal fluid. On the fetal side, PSβ_1G was just measurable in some samples of cord venous plasma and amniotic fluid, but none was detected in cord artery by our technique.

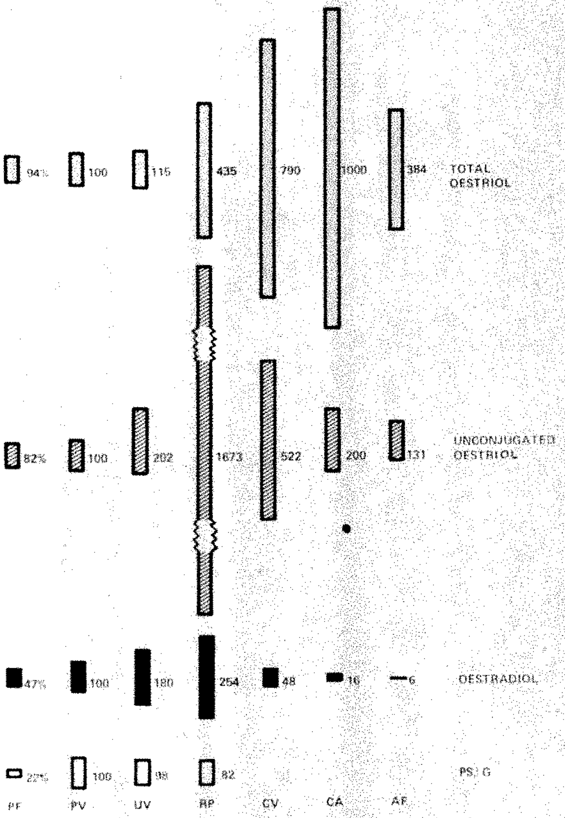

FIG. 1

Comparative distribution of oestrogens and PSβ_1G in maternal peritoneal fluid (PF) maternal peripheral vein (PV), uterine vein (UV), retroplacental blood (RP), cord vein (CV), cord artery (CA) and amniotic fluid (AF). The amount of oestrogen or PSβ_1G in maternal peripheral vein blood was assigned a value of 100 per cent and served as the basis for comparisons.

Oestriol

Retroplacental blood had the highest mean value of unconjugated oestriol with a progressive reduction in level to cord vein, cord artery and amniotic fluid. On the maternal side the order was uterine vein, peripheral vein, and peritoneal fluid.

The highest mean value of total oestriol was found in umbilical arterial plasma. Smaller mean values were found in cord vein and amniotic fluid. The mean values in retroplacental blood were smaller than in cord blood, although this still represented the highest concentration found in the maternal compartments. Values fell in order from retroplacental blood, to uterine vein, peripheral vein, and peritoneal fluid.

Oestradiol

Oestradiol was also found in highest concentration in retroplacental blood, and the pattern of diminution seen on either side of the placenta was similar to that seen with unconjugated oestriol, although the gradients were more acute.

In maternal peripheral blood, positive correlations were only found between unconjugated oestriol and oestradiol ($\rho = 0{\cdot}55$, $p < 0{\cdot}05$, $n = 17$) and total oestriol and PSβ_1G ($\rho = 0{\cdot}65$, $p < 0{\cdot}01$, $n = 17$). Correlations of PSβ_1G between various compartments where it was present in sufficient amounts to be measured by our techniques were calculated. Correlations between peritoneal fluid and peripheral vein, uterine vein and retroplacental blood were ρ 0·87 ($p < 0{\cdot}01$, $n = 12$), ρ 0·75 ($p < 0{\cdot}02$, $n = 10$) and ρ 0·67 ($p < 0{\cdot}01$, $n = 12$) respectively. The correlation between peripheral vein and uterine vein was 0·94 ($p < 0{\cdot}01$, $n = 17$) and retroplacental blood 0·70 ($p < 0{\cdot}01$, $n = 20$); between retroplacental blood and uterine vein it was 0·58 ($p < 0{\cdot}01$, $n = 16$). While mean values showed differences between compartments, there were individual exceptions. For example when peripheral vein values were high the difference between uterine vein and peripheral vein PSβ_1G values became less marked.

For unconjugated oestriol more compartments could be compared although fewer paired data were available for fetal compartments (see Table I). There was no significant correlation between peritoneal fluid values and uterine vein ($n = 11$) nor retroplacental blood values ($n = 13$). Peripheral vein values correlated significantly with the values in the following compartments, peritoneal fluid ρ 0·64 ($p < 0{\cdot}05$, $n = 12$), uterine vein ρ 0·44 ($p < 0{\cdot}05$, $n = 17$), retroplacental blood ρ 0·5 ($p < 0{\cdot}05$, $n = 14$), cord vein ρ 0·07 ($p < 0{\cdot}01$, $n = 14$) and cord artery ρ 0·09 ($p < 0{\cdot}05$, $n = 5$). Retroplacental blood showed a significant correlation with both uterine vein values $\rho = 0{\cdot}9$ ($p < 0{\cdot}01$, $n = 16$) and cord vein values $\rho = 0{\cdot}9$ ($p < 0{\cdot}01$, $n = 13$). Amongst the fetal compartments the numbers of results available were too small for useful comparison.

The data available for correlating values between the compartments for total oestriol were similar to oestriol and yet the only two significant correlations were between peripheral vein and peritoneal fluid ρ 0·72 ($p < 0{\cdot}01$, $n = 12$) and uterine vein ρ 0·38 ($p < 0{\cdot}01$, $n = 16$).

In spite of similar numbers of samples in the case of oestradiol significant correlations were found only between maternal vein values and values in uterine vein ρ 0·56 ($p < 0{\cdot}05$, $n = 16$) and retroplacental blood ρ 0·41 ($p < 0{\cdot}05$, $n = 16$). A significant negative correlation was found between uterine vein, oestradiol and cord artery ρ $-0{\cdot}9$ ($p < 0{\cdot}02$) but only six paired results were available.

DISCUSSION

Evidence that PSβ_1G is produced by the trophoblast has been adduced by Tatarinov and Masyukevich (1970), Bohn (1971), Lin (1976) and Horne *et al* (1976). The fetal compartments contain much less than the maternal compartments and it is evident that if the placenta is the point of origin, very little is passed to the fetus. The traces in the fetal compartments could be accounted for by a small inflow from maternal compartments without postulating any placental production. The distribution in the maternal compartment is difficult to reconcile with a placental origin. The highest concentration occurred in the peripheral venous blood, not in retroplacental or uterine vein blood. The retroplacental blood and the uterine vein compartments are so much smaller than the peripheral venous compartment that one would expect a much higher PSβ_1G concentration in these if the protein was entering the maternal compartments from the placenta. The fact that

PSβ_1G is present in interstitial and peritoneal fluid shows that it is capable of penetrating the vascular endothelium. Other barriers, such as that between blood and cerebrospinal fluid appear to be totally impenetrable within the sensitivity limits of our assay system.

It is not surprising that there are no correlations between the concentrations of PSβ_1G and unconjugated oestriol or oestradiol. Although it is presumed that all arise from the syncytiotrophoblast their biosynthesis proceeds along different lines. In this context it is more difficult to account for the positive correlation between total oestriol and PSβ_1G in peripheral blood. However, if it is remembered that a large proportion of the oestriol conjugates entering the maternal circulation do so from the maternal liver where unconjugated oestriol is conjugated, and if PSβ_1G is produced by the maternal liver, its common point of entry with oestriol conjugates makes the association of total oestriol and PSβ_1G more understandable.

By much the highest oestradiol concentration occurs in the retroplacental blood, supporting the contention that this is its main point of entry. By the time it reaches the uterine vein it has been diluted and the peripheral venous level is still lower. Impeded outflow of oestradiol from the vascular compartment is reflected in the lowered concentration in peritoneal fluid. This steroid makes its way into the cerebrospinal fluid in sufficient amounts to be measurable and it is possible that oestradiol exerts some physiological action in the nervous system during pregnancy. Oestradiol levels in the fetal compartment are much less than in the maternal. The gradient from umbilical vein to artery suggests that oestradiol in the fetal compartment originates from the placenta and is then extracted to some extent by the fetus. The concentration in amniotic fluid is low and this is likely to result from fetal urination with the possibility of flow across the membranes from the maternal compartment or placenta.

There were few correlations between oestradiol concentrations in the various compartments. On the fetal side the liver presents a substantial barrier between arterial and venous circulations which would account for the umbilical artery and vein difference. Such a barrier does not apply on the maternal side. The fact that the peripheral vein and peritoneal fluid compartments are not related suggests that egress from the vascular compartment is limited by the protein binding of oestradiol.

The pattern of distribution of unconjugated oestriol follows that of oestradiol and the same deductions can therefore be made about the point of origin and direction of flow through the various compartments. Since part of the biogenesis of oestriol takes place in the fetus (Diczfalusy and Mancuso, 1969) it is not surprising that the concentrations of this steroid are higher in the fetal compartment than the maternal compartments compared to the purely placental steroid, oestradiol. The large number of correlations between oestriol concentrations in various compartments, even across the placenta, is taken as evidence of its poor affinity for binding proteins compared to oestradiol.

The pattern of distribution of total oestriol is different. The highest concentration occurs in the fetal circulation, presumably mainly as sulphate. It is known that this congugate can, to some extent, make its way across the placenta without hydrolysis (Levitz *et al*, 1967). Although, as expected, the concentration of total oestriol falls after leaving the retroplacental circulation, there is, surprisingly, no gradient from uterine vein to peripheral vein. Total oestriol is present in relatively high concentration in peritoneal fluid reflecting the relatively easy flow of this unbound steroid from the vascular compartment.

The compartmental distribution of the substances we have studied would fit the following model: PSβ_1G is produced in the maternal liver and is trapped in the placenta. It finds its way to a considerable extent into the extravascular maternal space but does not penetrate the placental barrier except in trace amounts. Oestradiol and unconjugated oestriol are secreted by the placenta into both maternal and fetal compartments and make their way into all adjacent compartments, albeit passage across intercompartmental barriers is impeded, more so with oestradiol than oestriol. Oestriol conjugates, presumably mainly sulphates, are carried to the placenta in the fetal circulation. These are partly hydrolysed in their passage through the placenta. A further inflow of oestriol conjugates, mainly glucosiduronates, takes place by conjugation of the steroid in the maternal liver and gut.

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METABOLISM OF DIPHENYLHYDANTOIN (PHENYTOIN) DURING PREGNANCY

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Summary

Plasma levels of diphenylhydantoin (DPH) and the 24 hour urinary excretion of both DPH and its major metabolite 5-(p-hydroxyphenyl)-phenylhydantoin (HPPH) were measured serially during pregnancy in a group of five unselected epileptic patients. Plasma DPH levels fell and on average were some 40 per cent lower at term than they had been in early pregnancy. With one exception, the patients were well controlled during pregnancy but two patients who regularly had plasma DPH levels below 10 μg/ml both suffered seizures in the puerperium. There was no convincing change in the excretion of either DPH or HPPH which together accounted for less than half the administered dose. Gestational length exceeded 41 weeks in 4 of the 5 patients studied but all were delivered of normal healthy infants, of average birthweight, with no congenital defects or clotting disorders.

A RECENT survey of epileptic patients suggested that half of them may experience more fits during pregnancy (Knight and Rhind, 1975). A variety of reasons have been put forward to explain this tendency and recent reports suggest that plasma levels of anticonvulsants may be lower during pregnancy (Mygind *et al*, 1976; Lander *et al*, 1977) but surprisingly there are no published data on the metabolism of anticonvulsants. Pregnancy is characterized by general changes in absorption, excretion and metabolism and it would be surprising if drugs such as anticonvulsants were not themselves affected in consequence. We report here a study of one major anticonvulsant, diphenylhydantoin (DPH) (Phenytoin sodium, Epanutin, Dilantin), during pregnancy.

METHODS

Five epileptic patients, all on long term DPH therapy, were studied serially during pregnancy and the puerperium. They were seen regularly throughout pregnancy at a routine antenatal clinic but any questions relating to the management of their epilepsy were referred to a neurologist independent of the study. Brief details of the patients involved are given in Table I. With one exception (Patient 5) all remained well controlled throughout pregnancy and consequently their treatment remained unaltered.

Each patient collected all urine passed during the 24 hours preceding her routine antenatal

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TABLE I

Patients taking diphenylhydantoin (DPH) during pregnancy

Patient	Age (years)	Parity	Daily dose of DPH (mg)	Weight (kg)	Other drugs		
					Anticonvulsants		Haematinics
1	23	0 + 0	300	49·6	Phenobarbitone	30 mg thrice daily	None
2	27	0 + 2	300	56·3	Myseline	250 mg thrice daily	Iron only
3	17	0 + 0	200	74·9	Phenobarbitone	30 mg thrice daily	Iron and folic acid
4	22	1 + 0	100	51·9	Phenobarbitone	60 mg at night	Iron and folic acid
5	23	0 + 1	300	49·6	Phenobarbitone	30 mg thrice daily	None
5 (from 32 weeks gestation)			400		Carbamazepine	50 mg thrice daily	None

clinic visit into a polythene bottle containing a few drops of five per cent aqueous hibitane as preservative. The volume was subsequently measured and an aliquot stored frozen for later analysis. At the clinic, 10 ml of venous blood were collected into a heparinized tube at approximately the same time each visit, plasma was prepared as soon as possible by centrifugation and stored frozen. Similar samples of both maternal and cord blood were collected at delivery together with an aliquot of amniotic fluid and the placenta if available.

DPH was measured by the method of Barrett (1971) and its major metabolite, 5-(p-hydroxyphenyl)-phenylhydantoin (HPPH) by the method of Chang and Glazko (1970). Derivatives of both were formed by on-column methylation using tri-methylanilinium hydroxide. A Pye Unicam 106 gas chromatograph with flame ionisation detectors was used throughout. Glass columns, six feet by 0·25 inches were packed with 3 per cent OV 17 on Gas Chrom Q 80–100 mesh and operated at 225°C for DPH and 250°C for HPPH. Injection and detector temperatures were set at 300°C for both compounds and the carrier gas was oxygen free nitrogen flowing at 40 ml/minute. Using this system the coefficient of variation between assays was 6·5 per cent for DPH and 14 per cent for HPPH.

RESULTS

In the four patients whose treatment remained unchanged, plasma DPH levels fell during pregnancy. One week after delivery, plasma levels were still low and although eight weeks later they had risen they were, with one exception, still below the levels found in early pregnancy. There was considerable individual variation but in general each patient followed the trend suggested by the mean (Fig. 1).

Excretion of DPH in a 24-hour urine collection was small and on average less than one per cent of the daily administered dose was recovered. Again there was considerable individual variation and in two patients (No. 3 and 4) there were occasions when a 24-hour collection contained no detectable DPH (Fig. 2). There was no relation between urine DPH levels and either urine volume or glomerular filtration rate as measured by 24-hour creatinine clearance (Table II). In general plasma and urine levels of DPH reflected the dose of the drug prescribed.

On average, 38 per cent of the prescribed dose was recovered as the metabolite HPPH; the higher the dose of DPH the greater the amount of HPPH excreted per 24 hours but the percentage recovered decreased with increasing dose (Fig. 3). There was no convincing change in HPPH excretion during pregnancy and the renal losses were unrelated to either urine volume or glomerular filtration rate (Table II).

Patient No. 5 complained of 'funny turns' and 'dizzy spells' from about 24 weeks gestation although no classical epileptiform attacks were ever witnessed by the medical staff. Her daily phenytoin intake was increased empirically by 100 mg without noticeable effect on either the

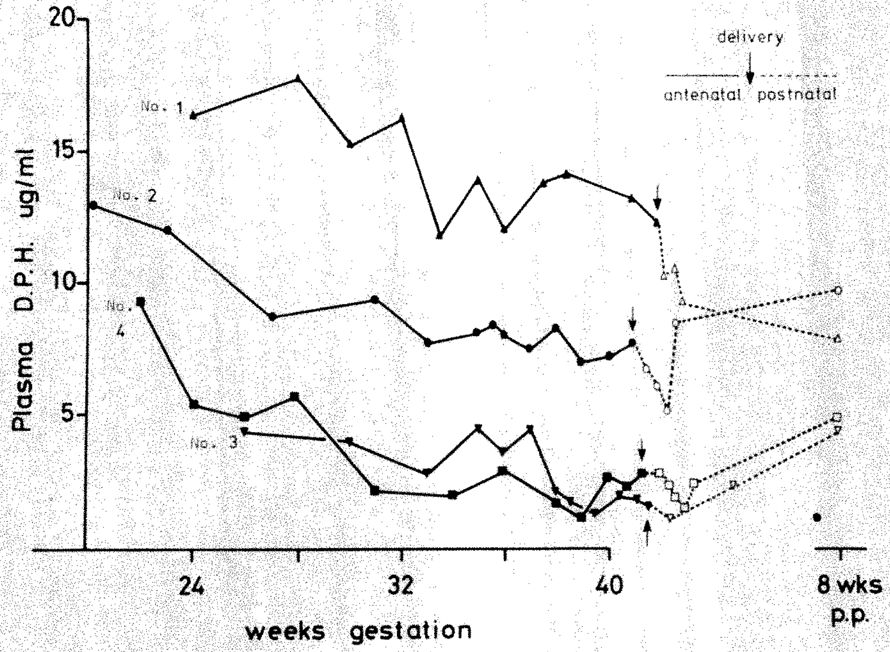

FIG. 1

Plasma levels of diphenylhydantoin during pregnancy and the puerperium.

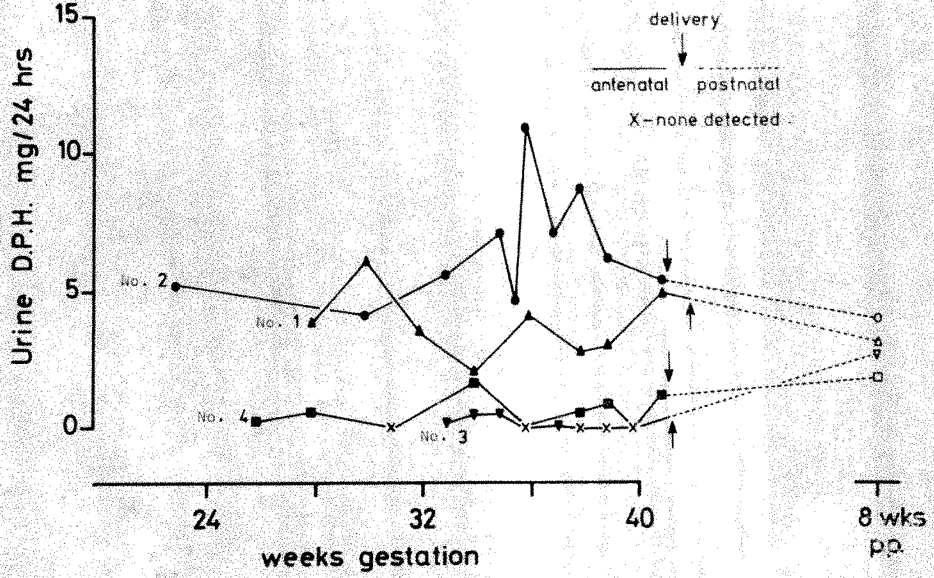

FIG. 2

Excretion of diphenylhydantoin during pregnancy and 8 weeks post partum.

TABLE II

Excretion of DPH during and after pregnancy by four patients whose treatment remained unchanged during and after pregnancy

	Gestation (weeks)	DPH (µg/ml) Plasma	DPH (µg/ml) Urine	HPPH (mg/l)	24-hour urine volume (ml)	HPPH total (mg)	Creatinine clearance (ml/minute)
Patient No. 1	28	13·0	2·0	34	1650	56	151
	30	15·5	3·5	56	1900	106	102
	32	16·5	3·0	58	1300	75	89
	34	14·5	0·5	44	1575	69	57
	36	12·0	2·5	54	1650	89	120
	38	14·5	2·5	78	1100	86	56
	41	15·5	3·0	60	1075	64	82
	42	14·5	3·5	72	1400	101	94
	8 weeks post partum	8·0	2·0	48	1550	74	92
Patient No. 2	23	12·0	4·0	73	1340	98	103
	31	9·5	1·5	81	1375	111	148
	33	8·0	2·5	54	2054	111	184
	35	8·5	3·5	54	2050	111	162
	36	8·0	5·0	75	2255	169	158
	37	8·0	4·0	59	1860	110	159
	38	8·5	4·5	51	1900	97	204
	39	7·1	4·0	67	1650	110	179
	40	7·5	1·0	58	1800	104	118
	8 weeks post partum	9·0	2·0	86	1950	168	125
Patient No. 3	33	3·0	0·5	64	950	61	122
	35	4·5	1·0	195	500	98	134
	36	3·5	1·0	64	1250	80	97
	37	4·5	nd	62	1375	85	96
	38	2·0	0·5	122	660	80	122
	39	1·5	nd	56	1275	71	87
	40	2·0	nd	62	1300	80	100
	41	3·0	nd	54	1450	78	117
	8 weeks post partum	4·5	2·0	88	2100	185	159
Patient No. 4	26	5·0	0·5	60	750	45	52
	28	6·0	0·5	37	1350	50	85
	31	2·0	nd	40	1130	45	87
	34	2·0	1·0	16	1690	27	86
	36	3·0	nd	36	1540	55	93
	38	2·0	0·5	31	1350	42	141
	39	1·0	0·5	28	1325	37	162
	40	3·0	nd	44	1440	63	141
	41	3·0	1·0	40	1500	60	112
	8 weeks post partum	5·0	1·5	26	1350	35	62

nd = none detected

plasma level of DPH or her symptomatology and a fortnight later carbamazepine was substituted for phenobarbitone (Fig. 4). Because of her history of poor control of epilepsy, this patient was induced at 38 weeks gestation by amniotomy and oxytocin.

The remaining patients in the group were allowed to go to term and all were delivered of normal healthy infants with neither congenital abnormalities nor bleeding disorders (Table III). Details of DPH levels at delivery are given in Table IV.

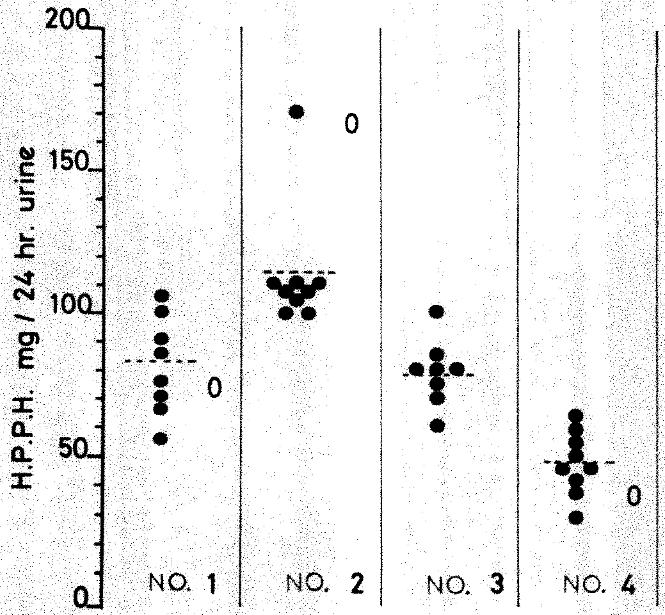

FIG. 3

Excretion of 5-(p-hydroxyphenyl)-phenylhydantoin (HPPH) during pregnancy (solid circles) and 8 weeks post partum (open circles). Horizontal pecked line represents mean excretion during pregnancy. Postpartum value for Patient No. 3 has been omitted because additional phenytoin was prescribed after delivery.

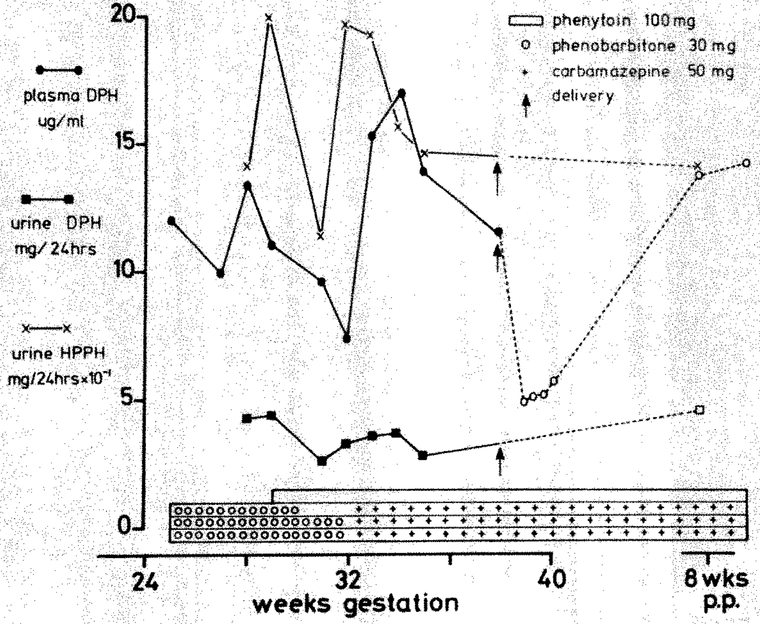

FIG. 4

Plasma levels of DPH and excretion of DPH and HPPH in Patient No. 5.

TABLE III

Details of delivery

Patient No.	Gestation (weeks)	Induction	Induction to delivery interval (hours)	Method of delivery	Birth weight (kg)	Sex
1	43	Yes	8¾	Spontaneous vertex	3·43	Male
2	41	No	(32¾)	Spontaneous vertex	3·20	Female
3	42	No	(17½)	Keillands forceps	3·42	Female
4	41	Yes	8¼	Spontaneous vertex	3·02	Female
5	38	Yes	4¾	Barnes forceps	3·48	Male

TABLE IV

Diphenylhydantoin levels at delivery

Patient No.	Maternal plasma (μg/ml)	Cord plasma (μg/ml)	Amniotic fluid (μg/ml)	Placenta (μg/g)	Placenta total (mg)
1	13·5	13·5	4·5	8·0	5·3
2	7·0	7·5	4·5	5·5	3·4
3	0·5	0·5	nd	6·5	4·4
4	3·0	2·0	3·5	10·0	5·6
5	11·5	7·5	1·5	17·5	14·3

nd = none detected

DISCUSSION

In every patient plasma levels of DPH fell below the commonly accepted therapeutic minimum of 10 μg/ml (Buchthal *et al*, 1960; Lund, 1974) at some point in this study. Although the advent of routine anticonvulsant assays has shown that in many patients effective control can be maintained with drug levels of less than 10 μg/ml (Reynolds *et al*, 1976) yet a drop in plasma concentration of some 40 per cent must compromise control in some patients. In fact, two patients in the present series (No. 3 and 4) suffered grand mal seizures in the immediate postnatal period, and in Patient No. 3 the daily intake of DPH was increased by 100 mg as a result.

Small differences in sampling time and time of medication may account for some of the individual variations in plasma DPH levels but we do not think that the falling plasma levels resulted from failure of the patients to take their prescribed medication. No patient defaulted, all were very co-operative and all were acutely aware of the danger to the fetus of a grand mal attack. Furthermore, there was no significant change in the excretion of HPPH over the period studied. The normal physiological increase in blood volume during pregnancy may in part be responsible for the reduced concentrations of DPH. However, assuming an average increase in plasma volume of 750 ml over the period studied, calculation of the total circulating mass of DPH still gives lower results as pregnancy progresses. An increase in the distribution space of some 6 l would be needed to entirely account for the fall in plasma concentration. The increase in the interstitial fluid volume during pregnancy may be of this order.

It seems unlikely that the renal losses of DPH made a significant contribution to the falling plasma levels in the present series. There was no convincing change in DPH excretion during or after pregnancy and in general the greater the dose of DPH prescribed the more DPH was excreted. It is difficult to draw conclusions from such a small series—only two patients (No. 1 and 2) were taking the same dose of Epanutin (300 mg per day). These two patients had significantly different mean plasma DPH levels but there was no significant difference in 24-hour DPH excretion, urine volume or glomerular filtration rate. There was, however, a significant difference in their excretion of HPPH which presumably reflected different rates of metabolism.

Presumably only 'free' DPH is filtered through the glomerulus and in non-pregnant subjects this may be only 10 per cent of the total in plasma. *In vitro* studies suggest that there is no significant change in the capacity of plasma proteins to bind DPH during pregnancy but if albumin is the major carrier protein then the falling plasma albumin concentration could increase the proportion of 'free' DPH (Hooper *et al*, 1974). If the 'free' DPH is the therapeutically effective fraction, then it may be significant that the two patients who suffered epileptic attacks in the puerperium excreted no DPH during a 24-hour urine collection on one or more occasions in the previous month. It seems reasonable to assume from this that there was little or no free DPH available for filtration at the glomerulus, that tissue levels had gradually become depleted and seizure control was eventually compromised. Excretion of DPH in 24-hour urine collections may therefore be a measure of the plasma levels of free DPH and as such serve as a useful clinical index of effective therapy. Indeed, examination of the data provided by Bochner *et al* (1973) supports this thesis: in their series of 16 patients there was a highly significant correlation between plasma free DPH and DPH excreted in a timed urine collection. The concentration of DPH in a saliva sample has also been used as a measure of 'free' plasma DPH levels but a urine collection has the advantage that it can effectively 'average' the free plasma DPH level over a 24-hour period.

It was anticipated that changes in metabolism would amost certainly contribute to the altered picture of DPH levels during pregnancy. A number of studies have demonstrated similarities between enzyme systems which hydroxylate steroids and those which oxidise drugs: steroid hormones may well be the natural substrate for drug metabolizing enzymes of the hepatic microsomes (Kuntzman, 1969). Direct evidence in the case of DPH is scanty. In general, epileptic women appear to need higher doses of anticonvulsants than men to achieve effective control (Travers *et al*, 1972) and there are isolated reports of the failure of oral contraception amongst these patients. The administration of oestrogens to healthy male volunteers can reduce the half-life of acutely administered DPH (Fernandez-Pol and Zaninovich, 1975). Metabolism of DPH during pregnancy may be considerably influenced by the high levels of endogenous steroids at this time. It was noticeable that in the group studied, plasma levels of DPH eight weeks after delivery were still lower than they had been in early pregnancy. The possibility exists that in some individuals early pregnancy is associated with a rise in plasma DPH levels because of competition from endogenous steroids for the main metabolic pathways. This may be particularly relevant to the incidence of congenital abnormalities where high levels of DPH have been implicated (Richens and Houghton, 1973; Loughnan *et al*, 1973). A high capacity to handle steroids which persists in the early puerperium may explain the low plasma levels of DPH found in some patients at this time, for example plasma levels in Patient No. 5 fell by over 50 per cent within 24 hours of delivery.

If the excretion of HPPH is a realistic index of DPH metabolism during pregnancy, then there does not appear to have been any significant change throughout the period studied. HPPH is excreted via the kidney and the enhanced renal function characteristic of pregnancy would be more than adequate to handle any increase in HPPH production. Admittedly the percentage of the administered dose recovered as HPPH is rather lower in this series than has been reported in the literature for non-pregnant subjects (Glazko *et al*, 1969). Traditionally, low HPPH excretion and falling plasma DPH levels would suggest poor patient compliance but it may be that either malabsorption of this drug in pregnancy is rather more widespread than the one case reported by Ramsay *et al* (1978) would indicate or that the metabolism and disposition of this drug is not as straightforward as generally assumed. Unfortunately no data are available on the excretion of HPPH either during the puerperium or in very early pregnancy so the possibility of abrupt changes in metabolism at these times remains conjectural.

Whatever the explanation, data from the present study suggest that plasma DPH levels may fall by some 40 per cent during pregnancy and that patients may be particularly at risk during the immediate post-delivery period. Because of wide individual variations in DPH metabolism it is difficult to draw general conclusions about the management of epileptics during pregnancy. If it is realistic to apply the nomogram of Richens and Dunlop (1975) to this situation then an additional 50 to 100 mg DPH per day may be sufficient to compensate for the changes observed in pregnancy. Such additional medication may, however, produce toxic levels in the puerperium. Careful supervision of the patient and regular monitoring of plasma drug levels are obviously indicated.

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THE EFFECT OF OXYTOCIN IN INDUCED LABOUR ON NEONATAL JAUNDICE

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Summary

A prospective study in 180 mothers and babies examined the effects of oxytocin in induced labour on plasma bilirubin levels in cord blood, as well as on the incidence of neonatal jaundice. Raised plasma bilirubin levels in cord blood, probably enhanced by breakdown of fetal red cells, appeared to be a dose dependent effect of oxytocin. Commensurate with this was the finding that a larger proportion of babies in the induced group manifested a greater severity of jaundice.

THE association of induced labour and neonatal jaundice is still a subject of controversy (McConnell *et al*, 1973; Calder *et al*, 1974; Gould *et al*, 1974; Davies *et al*, 1974; Beazley and Alderman, 1975; Chalmers *et al*, 1975; Thiery *et al*, 1975; Chew, 1977; Friedman *et al*, 1978). Consequently, there has been much speculation about the effects of oxytocin in induced labour, but the cause of the jaundice remains obscure. Induced labour may be associated with preterm deliveries, instrumental deliveries, or epidural analgesia. Moreover, there is some uncertainty whether or not oxytocin crosses the placenta. In a retrospective study at St Mary's Hospital, Manchester, of a group of 4247 babies born between 1st January, 1971 and 31st December, 1971, the incidence of neonatal jaundice was approximately doubled in babies born following induced labour. The incidence of neonatal jaundice was 23 per cent when labour was induced but only 11 per cent when labour had started spontaneously. We were, therefore, prompted to carry out the present study to explain this increased incidence of neonatal jaundice.

METHODS

A group of mothers who had normal pregnancies, and were 40 to 43 weeks pregnant, were randomly selected and studied prospectively. Gestational age, calculated from the first day of the last menstrual period, was checked by reference to uterine size, fetal biparietal diameter, or fetal ossification centres. Rhesus negative mothers were excluded. In 90 mothers labour had been induced by amniotomy and oxytocin infusion. These mothers were matched with 90 control mothers of similar maternal age, gravidity, gestational age (40 to 43 weeks), smoking habits, and social class, in whom labour had started spontaneously. A similar proportion of mothers in both groups had previously used oral contraceptives. All 180 mothers gave birth by normal cephalic vaginal delivery; three others that gave birth by instrumental delivery were excluded from the study.

Labour in all 180 mothers was supervised by one person (P.B.) who also assisted at delivery. In induced labours the rate of oxytocin infusion, 10 units of oxytocin in 5 per cent (w/v) dextrose, was controlled by IVAC pumps (IVAC, UK, Harrow, Middlesex) starting at 2 drops per minute. The rate of infusion was doubled every 30 minutes until labour was established and was held constant at this rate thereafter. The total dose of oxytocin administered was recorded.

The umbilical cord was clamped within 60 seconds of birth. The clinical condition of the newborn babies was assessed by a subset of the Apgar scoring method (D'Souza *et al*, 1975). Scores of 0, 1 or 2 were given respectively when respiration was absent, gasping, or regular; when heart rate was absent, <100, or >100 beats per minute; or when colour was white, blue, or pink. Thus, each baby could score a maximum of 6 or a minimum of 0. All babies received routine care, and also 0·5 mg phytomenadione intramuscularly within three hours of birth. They were examined by a paediatrician within 24 hours of birth, and any evidence of trauma was recorded. Progress in the lying-in wards, and whether they were breast or bottle fed, was also recorded.

Umbilical arterial, and venous, blood were collected immediately after the cord was clamped and cut. Blood pH was measured by Astrup apparatus. Blood samples were collected in containers with 1 mg potassium EDTA and the haematological values were measured by a Coulter Counter, Model S (Coulter Electronics Ltd.). Plasma Na^+, K^+ and total (conjugated and non-conjugated) bilirubin levels were measured, in blood samples collected in heparinized containers, by standard auto-analyzer methods. In the neonatal period, blood from jaundiced babies obtained by heel-prick was collected into plain containers; total serum bilirubin was measured by spectrophotometry.

RESULTS

Ninety mothers, induced in labour, gave birth to 46 female and 44 male babies; the birth weight (mean ± SE) was 3388±41 g (range 2580 to 4040 g). The 90 control mothers, who started labour spontaneously, gave birth to 49 female and 41 male babies; their birth weight was 3372±51 g (range 2515 to 4280 g). There was no statistical difference between the birth weights of the two groups.

Haematological values in cord blood

The haematological values in cord blood in the induced and the spontaneous labour groups are shown in Table I. Unfortunately, a small proportion of results failed to be processed by the laboratory. There was a significant reduction in cord blood haemoglobin (P <0·01), in the haematocrit (P <0·001), and in the red blood cell (RBC) count (P <0·01) in the induced group. The reduction in mean cord blood haemoglobin, in the haematocrit, and in the RBC count was of the order of 6 to 7 per cent.

Plasma Na^+, K^+ and bilirubin levels in cord blood

The mean levels of plasma Na^+, K^+ and Na^+/K^+ ratio in cord blood in the induced and the spontaneous labour groups were similar (Table II); but the mean plasma bilirubin level was

TABLE I

Haematological values in cord blood

Cord blood	Induced labour Mean ± SE (n)	Spontaneous onset of labour (Controls) Mean ± SE (n)	'P'
Haemoglobin (g/dl)	16·1 ±0·3 (83)	17·1 ±0·2 (86)	<0·01
Haematocrit	0·484 ±0·009 (83)	0·522 ±0·007 (86)	<0·001
Red blood cells ($\times 10^{12}$/l)	4·55 ±0·08 (83)	4·88 ±0·07 (86)	<0·01

TABLE II

Plasma Na$^+$, K$^+$, Na$^+$/K$^+$ ratio, and bilirubin levels in cord blood

Cord blood	Induced labour Mean ± SE (n)	Spontaneous onset of labour (Controls) Mean ± SE (n)	'P'
Plasma Na$^+$ (mmol/l)	137±0·43 (84)	136±0·5 (84)	NS
Plasma K$^+$ (mmol/l)	4·6±1·0 (84)	4·5±1·0 (84)	NS
Plasma Na$^+$/K$^+$ ratio	31±1·0 (84)	30±1·0 (84)	NS
Plasma bilirubin (μmol/l)	37±1·0 (90)	30±1·0 (90)	<0·000001

NS = not significant

TABLE III

Effects of perinatal conditions on plasma bilirubin levels in cord blood

	Bilirubin levels (μmol/l)		
Perinatal conditions	Induced labour Mean ± SE (n)	Spontaneous onset of labour (Controls) Mean ± SE (n)	'P'
Gestational age (*weeks*)			
<41	36±2 (23)	30±2 (36)	<0·05
41–42	36±1 (59)	33±1 (47)	<0·05
>42	39±4 (8)	32±5 (7)	NS
Birth weight (*g*)			
<3000	37±2 (15)	33±1 (24)	NS
3000–3500	37±1 (41)	30±2 (29)	<0·01
>3500	34±1 (34)	32±2 (37)	NS
Modified Apgar scores			
<4	38±2 (17)	34±3 (25)	NS
5	35±1 (55)	31±1 (48)	<0·01
6	35±2 (18)	31±2 (17)	NS
Cord venous blood pH			
<7·3	36±2 (17)	30±2 (15)	<0·05
7·3–7·4	36±1 (46)	32±1 (40)	<0·01
>7·4	37±2 (18)	32±3 (15)	NS
Cord arterial blood pH			
<7·25	35±1 (31)	32±2 (26)	NS
7·25–7·30	38±2 (17)	32±2 (21)	<0·05
>7·30	37±2 (23)	33±2 (19)	NS
Length of labour (*hours*)			
<6	36±1 (58)	30±2 (12)	<0·01
6·12	37±2 (24)	31±2 (50)	<0·05
>12	34±3 (8)	31±2 (28)	NS
All patients	37±1 (90)	30±1 (90)	<0·000001

significantly ($P < 0\cdot000001$) raised in the induced group. The mean plasma bilirubin level in the induced group was 23 per cent higher than in the spontaneous labour group.

Effects of perinatal conditions on plasma bilirubin levels in cord blood

The effects of gestational age, birth weight, birth scores, cord venous blood pH, cord arterial blood pH, and length of labour, on plasma bilirubin levels in cord blood were examined. The results are shown in Table III.

The distribution of gestational ages was similar in the induced and in the spontaneous labour groups. There was no correlation between gestational age and plasma bilirubin levels. When plasma bilirubin levels in the induced or the spontaneous labour groups were compared the differences reached statistical significance at less than <41 weeks ($P < 0\cdot05$), or at 41 to 42 weeks ($P < 0\cdot05$) gestational age.

The babies were divided into three subgroups by birth weight. The distribution of birth weights less than 3000 g, 3000 to 3500 g and more than 3500 g was similar in the induced and the spontaneous labour groups. There was no correlation between plasma bilirubin levels and birth weight. Differences in plasma bilirubin levels between the induced and spontaneous labour groups reached statistical significance ($P < 0\cdot01$) in the 3000 to 3500 g birth weight group.

The babies were also analyzed to take into account the severity of birth asphyxia as assessed by birth scores, cord arterial blood pH, and cord venous blood pH. The distribution of birth scores, as well as of cord arterial or venous blood pH values, was similar in the induced as compared with the spontaneous labour group. Therefore, there was no difference in the severity of asphyxia in the two groups. Differences between the two groups in plasma bilirubin levels reached statistical significance when the birth score was 5 ($P < 0\cdot01$), when cord venous blood pH was <7·3 ($P < 0\cdot05$), or 7·3 to 7·4 ($P < 0\cdot01$), or when cord arterial blood pH was 7·25 to 7·30 ($P < 0\cdot05$). There was no correlation found between plasma bilirubin levels and birth scores, cord arterial blood pH, or cord venous blood pH.

As expected, a statistically greater proportion of mothers in the induced group ($P < 0\cdot000001$) were in labour for a shorter period. Fifty-eight (64 per cent) of 90 mothers induced were in labour for less than 6 hours as compared with 12 (13 per cent) of 90 who started labour spontaneously. Significantly higher levels of bilirubin in cord blood were noted in the induced group when labour lasted less than 6 hours ($P < 0\cdot01$) or 6 to 12 hours ($P < 0\cdot05$).

The drugs received during labour for pain relief and sedation were pethidine, phenergan, and a nitrous oxide/oxygen mixture (Entonox). None of the mothers received an epidural block.

Plasma bilirubin levels in cord blood in relation to the total dose of oxytocin administered during labour

The sample studied was broken down into four groups according to the total dose of oxytocin administered during labour (Fig. 1). The plasma bilirubin level in cord blood

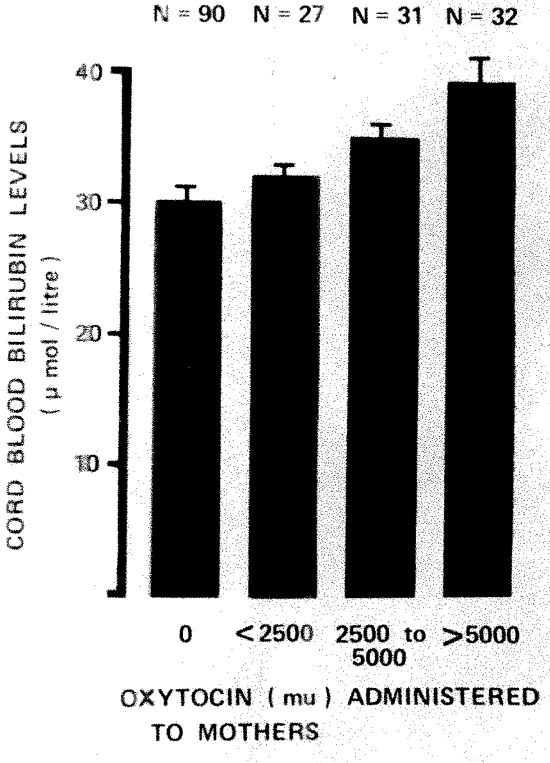

FIG. 1

Cord blood bilirubin levels (mean ± SE) at birth in relation to the total dose of oxytocin in induced labour.

(mean ± SE) increased from 30 ± 1 μmol/l in mothers receiving no oxytocin since labour started spontaneously, to 39 ± 2 μmol/l in mothers who had received more than 5000 mU (5010 to 27360 mU) of oxytocin; a difference of 30 per cent ($P < 0{\cdot}001$). A 15 per cent increase in plasma bilirubin levels in cord blood was also noted in mothers who had received a total dose of 2500 to 5000 mU of oxytocin ($P < 0{\cdot}05$); but the plasma bilirubin levels were not statistically different in mothers who had received less than 2500 mU (22 to 2400 mU) of oxytocin.

The regression correlating plasma bilirubin levels in cord blood to the total dose of oxytocin administered during labour is given by the equation:—

$$\text{cord plasma bilirubin} = 0{\cdot}000873 \times \text{number of mU of oxytocin} + 32{\cdot}2$$

The correlation between the cord plasma bilirubin and the total dose of oxytocin was $r = 0{\cdot}47$ ($P < 0{\cdot}000001$). The SE of the regression coefficient b was 0·00018.

Neonatal jaundice

There was a similar incidence of neonatal jaundice in the induced and spontaneous labour groups; 22 of 90 babies in the induced and 20 of 90 babies in the spontaneous labour group had jaundice in the first three postnatal days. Serum bilirubin was measured once or twice in 24-hour periods after jaundice was diagnosed until bilirubin levels started to decline. The frequency of various serum bilirubin levels is shown in Table IV. A significantly ($P < 0{\cdot}05$) greater proportion of jaundiced babies in the induced group had serum bilirubin levels above 200 μmol/l. Ten of 22 jaundiced babies in the induced group and only 4 of 20 babies in the spontaneous labour group had bilirubin levels above 200 μmol/l.

TABLE IV

Maximal serum bilirubin levels in jaundiced babies

Labour	N	Serum bilirubin levels (μmol/l) <100	100–200	>200
Induced	22	8	4	10*
Spontaneous	20	15	1	4
Total	42	23	5	14

* $\chi^2 = 3{\cdot}93$, $P < 0{\cdot}05$

There was no cause found for the hyperbilirubinaemia in the jaundiced babies. In view of the association between breast-feeding and neonatal jaundice the effect of the type of feeding on the incidence of neonatal jaundice, or on the maximal level of serum bilirubin, was examined but none was found. The frequency of breast or of bottle feeding was the same in both sets of babies. In the induced group, one baby had oral thrush and one had undescended testes. In the spontaneous labour group one baby had oral thrush and one had conjunctivitis.

Discussion

Our study has shown that labour induced by amniotomy and oxytocin infusion is associated with raised levels of plasma bilirubin in cord blood. Moreover, there was a relation between the total dose of oxytocin administered to mothers and the level of plasma bilirubin in cord blood. Proof of causality must obviously rest on the kind of mothers selected for induction. As far as we were able to ascertain, the two groups of mothers studied were similar in respect of maternal age, gestational age at delivery, gravidity, smoking habits, use of oral contraceptives before pregnancy, and social class. The association between induced labour and raised plasma bilirubin levels in cord blood still held when the data were analysed by gestational age, birth weight, birth scores, pH of cord arterial or venous blood, or length of labour.

In the present study, the haematological findings in cord blood rule out the possibility of increased placental transfusion in induced labour. There is a suggestion from our results that in induced labour there is an increased loss of fetal red blood cells (Table I). Increased levels of plasma bilirubin in cord blood may therefore reflect an increased rate of red cell breakdown and an enhancement of haemoglobin catabolism. This implies that induced labour is in some way damaging to fetal red cells. One cause for such damage may be trauma due to the contracting uterus under the influence of oxytocin acting directly on the fetus from the onset of labour. By contrast, in spontaneous labour, amniotic fluid acts as a buffer between the fetus and the uterine wall. In our control mothers, the membranes ruptured near the end of the first stage or in the second stage of labour.

The possible role of hypoxia and acidosis in influencing bilirubin metabolism by displacing bilirubin from albumin-binding sites was investigated by examining the effects of birth scores, and of cord arterial or venous blood pH. The distribution of birth scores, as well as of pH values were similar in both sets of babies, but in similar subgroups higher levels of plasma bilirubin in cord blood were related to induced labour (Table III). Thus, the association between oxytocin induced labour and raised plasma bilirubin levels persisted irrespective of fetal condition, including acidosis, at birth.

The inconsistency in results of several studies on the relation of oxytocin induction and hyperbilirubinaemia in the neonatal period is well known but when a relation is shown, as in three studies (Calder *et al*, 1974; Davies *et al*, 1974; Sims and Neligan, 1975), the explanation given is reduced activity of hepatic enzyme systems involved in bilirubin metabolism; why an excess of such babies occurred in those studies but not in others (McConnell *et al*, 1973; Gould *et al*, 1974; Thiery *et al*, 1975) which cannot confirm this relation has not been adequately explained. Since mothers were randomly selected for induction, it is valid to assume that the frequency of babies with immature liver function should be the same in all six studies. A better explanation for the increased frequency of neonatal hyperbilirubinaemia lies in the effect of the total dose of oxytocin administered in induced labour. Our study has shown that higher doses of oxytocin administered are reflected by higher levels of plasma bilirubin in cord blood.

Since induced labour may produce a 20 per cent increases in plasma bilirubin levels at birth, such babies are faced with having to deal with larger amounts of bilirubin in the early neonatal period. The clinical implications of this may be important in at least two situations. In premature babies, because of lower concentrations of plasma albumin, a tendency to acidosis due to birth asphyxia, apnoeic episodes, and respiratory distress, there may be a greater risk of bilirubin being displaced into tissues. Secondly, in babies affected by haemolytic disease, haematin displaces bilirubin from albumin binding sites resulting in higher tissue concentrations of bilirubin than might be expected with comparable levels of plasma bilirubin not accompanied by haemolysis (Oski and Naiman, 1972). Clearly, this is not an area for complacency since oxytocin is widely used in present-day obstetric management.

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FURTHER CLINICAL EXPERIENCE IN THE TREATMENT OF HIRSUTISM WITH CYPROTERONE ACETATE

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Summary

Further experience in the treatment of female hirsutism with the anti-androgen cyproterone acetate is reported. Two-thirds of treated patients have shown improvement but relapse occurred within two or three months of stopping treatment. Acne also healed in two patients.

IN 1977 we reported our initial experience in the treatment of 14 patients suffering from idiopathic hirsutism or polycystic ovarian disease with hirsutism, using cyproterone acetate (Dewhurst *et al*, 1977). Ten of the 14 patients were improved and severe acne was healed in one patient. We now report further experience with this treatment and a study of patients after stopping treatment.

PATIENTS, TREATMENT AND RESULTS

Twenty-seven patients have been treated. Initial clinical and biochemical screening was done to make a diagnosis and to exclude ovarian tumours, adrenal tumours or adrenal hyperplasia. The clinical features and the diagnosis are shown in Table I. Polycystic ovarian disease was diagnosed only if the ovaries had been seen at laparoscopy or laparotomy.

An assessment of the severity of the hirsutism and the results of treatment was done using the total body hair scoring system of Ferriman and Gallwey (1961) and also by comparing photographs of the patient before and after every six months of treatment. The patients' subjective response to treatment was also noted.

The dosage regimen of Hammerstein and Cupceancu (1969) was used. This regimen inhibits ovulation and maintains a menstrual cycle. The initial dosage in all patients was 100 mg of cyproterone acetate daily from day 5 to day 14 of the menstrual cycle and 0·05 mg of ethinyl oestradiol from day 5 to day 25 of the cycle. This treatment was adhered to by 15 patients for 6 months, by 10 patients for 12 months and 2 patients for 18 months. The daily dosage of cyproterone acetate was increased to 200 mg in three patients who showed no improvement on the initial dosage and also in one patient whose improvement was slight. The dosage was reduced to 50 mg of cyproterone acetate after six months of treatment in three patients. Additional contraceptive precautions were advised in these three patients. Cosmetic removal of hair was permitted in all patients. The results of treatment are shown in Table II and the hair scores before and after treatment are shown in Table III. Improvement was sometimes delayed for up to three months on treatment. The earliest subjective signs of improvement were that hair growth was slower and that the hair was paler and finer than before (Fig. 1). Then the area of skin covered by hair decreased in size. Successful treatment was associated with a decrease in hirsutism which occurred in all areas of the body except the scalp, which was usually unchanged; when temporal recession of hair had occurred, it grew again with treatment. One patient lost a little head hair during treatment (see side effects). Acne improved markedly in two patients.

If a patient showed no improvement after six months of treatment, further treatment at the same dosage or at an increased dosage had no effect. Improvement was moderate in some patients and dramatic in others. A dosage reduction in three patients was followed by a relapse in one of them.

Treatment was continued for six months or until it was thought maximum benefit had been obtained. Eleven patients were followed after stopping treatment. Relapse occurred within two or three months in ten of them. Their hair scores are shown in Table III. Four of the

TABLE I

Clinical features and diagnosis of 27 patients

Age		
Mean 30 years		
Range 16 to 52 years		
Marital State		
Single	15	(2 had been pregnant)
Married	10	(7 parous, 4 infertile)
Divorced	2	
Hirsutis		
Duration	Mean 11 years	
	Range 1–35 years	
Area affected	Face 26	
	Trunk 12	
	Limbs 21	
Diagnosis		
Hirsutism + polycystic ovarian disease		8
Hirsutism + oligomenorrhoea		7
Hirsutism and normal periods		11
Postmenopausal		1
Total patients		27

TABLE II

Results of treatment according to diagnosis

Diagnosis	No. of patients	Improved with treatment No.	Improved with treatment Per cent
Hirsuties with polycystic ovarian disease	8	5	62·5
Hirsuties with oligomenorrhoea	7	5	71·4
Hirsuties with normal periods	11	7	63·6
Postmenopausal	1		
All patients	27	18	66·7

TABLE III

Hair scores before and after treatment

Time of assessment	Hair scores Mean	Hair scores Range
Initial	20	11–31
After 6 months	14	4–28
After 12 months	13	4–24
3 to 6 months after end of treatment	18	12–25

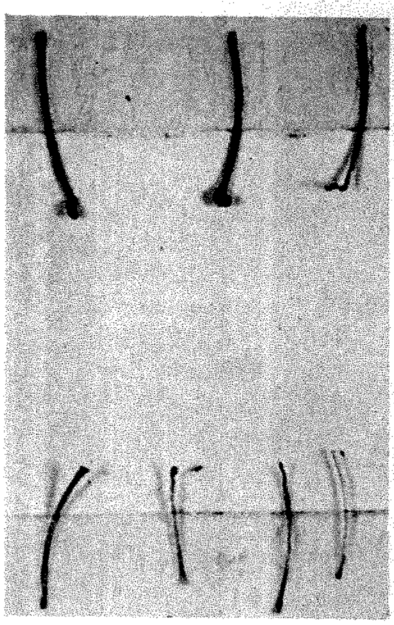

FIG. 1

The appearance of hair before (above) and after (below) six months' treatment with cyproterone acetate.

patients with recurrent hirsuties were taking oral contraceptives at the time. Nine patients had a second course of treatment after an interval of three months to one year. Five have completed six months and one has completed a year of further treatment and all are improved again.

Various side effects were seen. Five patients complained of tiredness but none wished to stop treatment. Three patients noted weight gain but all responded to diet. Two menopausal patients were cured of hot flushes. One patient who had shown no improvement was depressed and another was emotionally labile. One patient developed galactorrhoea and treatment was stopped; one lost some head hair. In one patient thyrotoxicosis was diagnosed while on treatment. Mental attitudes were difficult to assess but several patients with moderate hirsutism became less concerned about their hirsutism and better able to cope with cosmetic removal of hair following hormone therapy.

The plasma levels of testosterone, androstenedione and 5α dihydro testosterone did not alter significantly with treatment.

DISCUSSION

These results confirm our previous findings of the effect of cyproterone acetate in hirsute women with two thirds of our patients improved on treatment. In view of the incidence of side effects, notably weight gain and tiredness, we attempted to find the minimal effective dosage of the drug, but dosage reduction did lead to relapse in one patient. The effect of stopping treatment was disappointing in that relapse usually occurred necessitating further treatment. However, not all patients had a second course of treatment; reasons for this included the desire for a pregnancy or the improved mental state of the patient, whereby she was prepared to accept local cosmetic measures only for her hirsutism. Some patients went abroad where treatment was not available. Where treatment was resumed, improvement recurred, and tachyphylaxis was not seen.

Like Anderson and Browning (1977), we found no significant fall in plasma androgens while on treatment, although Ebling *et al* (1977) and Frölich *et al* (1977) reported a fall in androstenedione levels with a slight reduction in testosterone levels. However, it seems likely that the major effect of the drug on hair growth is due to competition with androgens at the hair receptors of the hair follicles. The result of this effect is to slow hair growth, decrease hair diameter and decrease pigment (Fig. 1) (Ebling *et al*, 1977).

Sebaceous secretion is also reduced (Ebling *et al*, 1977) and results in the healing of acne.

The cosmetic removal of hair using a razor, depilatory creams, tweezers and electrolysis have been the mainstay of treatment for the majority of hirsute women. Many people fear that removing hair makes it grow again more quickly but this fear is ungrounded (Peereboom-Wynia, 1972). Cyproterone acetate undoubtedly could have serious teratogenic effects if taken during pregnancy, and has caused feminization of male rat fetuses (Hamada *et.al*, 1963). It has also been shown to suppress ACTH and cortisol (Girard and Bauman, 1975; Jeffcoate *et al*, 1976), although overt signs of adrenal insufficiency have not so far been reported (Leading article, 1976).

The treatment is only effective while the drug is actually being administered. An intermittent regime with a break from treatment for a few months every year is perhaps advisable when long-term treatment is required but the possible side effects of prolonged therapy are uncertain.

Cyproterone acetate is not yet available for general use in the United Kingdom.

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TREATMENT OF PREMENSTRUAL SYNDROME BY SPIRONOLACTONE

BY

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Summary

Spironolactone was given to 28 women in a double blind cross over trial during four menstrual cycles. Hormonal profiles were measured during the first two cycles. Plasma aldosterone was elevated in the premenstrual phase of the cycles but there was no significant difference between symptomatic and asymptomatic groups. The rise in serum progesterone was higher in the symptomatic group during the postovulatory phase. The administration of spironolactone reduced weight and relieved psychological symptoms in more than 80 per cent of the symptomatic group.

THE occurrence of mood disturbance (Frank, 1931) and physical symptoms (Sweeny, 1934) associated with the menstrual cycle has been recognized for many years. Although a correlation between these symptoms and fluid retention has been described (Janowsky *et al*, 1973), a satisfactory explanation of their mechanism is wanting. The response to existing forms of treatment is variable despite the many published claims.

Renin (Brown *et al*, 1964; Skinner *et al*, 1969), angiotensin II (Sundsfjord and Aakvaag, 1970) and aldosterone (Katz and Romfh, 1972) are elevated during the luteal phase of the normal menstrual cycle. Since aldosterone is the most potent naturally occurring sodium-retaining hormone known, it seems logical to postulate aldosterone as a causative agent in the premenstrual syndrome.

This study aimed to test that hypothesis by determining aldosterone levels in symptomatic and asymptomatic subjects and to evaluate the efficacy of the aldosterone antagonist, spironolactone.

METHODS

Study group

The study group consisted of volunteers from the hospital nursing, technical and physiotherapy staff. Both symptomatic and asymptomatic volunteers were accepted, but those with a history of gynaecological, renal, hepatic or hypertensive disease and those taking oral contraceptives were excluded.

There were 28 subjects who were studied over a total of 106 menstrual cycles, in a double-blind cross over trial of spironolactone (Aldactone ®); 25 volunteers completed four cycles, while 3 completed two. The cycle was divided into menstrual, preovulatory, post-ovulatory and premenstrual phases. The duration of the menstrual phase varied with cycle length whilst the other phases were each of seven days, dating backward from the first day of the next menstrual period. Blood samples were taken in each phase of the first two cycles on days 4, 12, 18 and 24 and assayed for proprogesterone and aldosterone. Daily measurement of mood and weight was made throughout the study.

Treatment

Subjects were given 25 mg of spironolactone (Aldactone ^{R}) or a placebo four times daily from day 18 to day 26 of each of four cycles, the cycles having been randomized by allocation to four groups in a double-blind cross-over manner.

Thus, for each subject, the first two cycles in which hormone assays were performed, consisted of one treated and one placebo cycle. Subjects were randomly allocated to four groups, such that the order of treatment and placebo cycle was varied.

Mood assessment

Daily self-assessment of mood was made using visual analogue scales (Aitken, 1969). From this, the Premenstrual Mood Index was calculated as follows. A mood chart was devised comprising visual analogue scales for eight commonly occurring mood types: depression, sadness, tension, feeling of bloatedness, loss of libido, aggression, lethargy, and anxiety. The subjects marked each 100 mm scale at the same time each day, at a point along the line appropriate to their mood. The score was determined by measuring the distance in mm, this being proportional to symptom change. The total score was calculated for each day. The difference of the preovulatory from the premenstrual phase-mean gave the Premenstrual Mood Index. This provided a single figure estimate of mood change for each cycle: a positive score indicating a symptomatic cycle.

Since the subjects who considered they did not have premenstrual syndrome did experience between one and seven symptoms, definition of affected and control groups was made on the basis of the Premenstrual Mood Index. Only those with a positive Premenstrual Mood Index in all placebo cycles were considered the symptomatic group, those with inconsistent change the control group.

Weight

Weight was recorded by each subject, every morning immediately after rising and passing urine. The mean was calculated for each phase.

Hormone assays

Plasma aldosterone levels were measured by radioimmunoassay using antiserum 088 donated by the National Institutes of Health (Bethesda). Plasma extracts were purified by paper chromatography in the system hexane:toluene: methanol:water—1:9:5:2·5 v/v. The limit of detection of aldosterone from the curve was 5 pg. Replicate assays on pooled plasma gave 71·1±4·0 ng/100 ml (mean±SEM) with a coefficient of variation of 14·8 per cent between assays and 9·9 per cent within assays. There was no cross reaction with spironolactone.

Progesterone levels were measured by radioimmunoassay using antiserum raised in rabbits against an 11-conjugate coupled to rabbit serum albumin, after being extracted with hexane (Kutas *et al*, 1972; Furuyama and Nugent, 1971). Standards were assayed in triplicate; samples in duplicate. Replicate assays on commercial control serum (Ortho diagnostic controls III) gave 6·29±0·736 nmol/l (mean±SD) with a coefficient of variation of 11·69 per cent between assays and 9·06 per cent within assays.

Results were compared using Wilcoxon's signed rank test for paired data; the Mann-Whitney test for comparing the two groups and Spearman's correlation coefficient for assessing correlation between mood change and hormonal change.

RESULTS

Eighteen subjects were defined as symptomatic according to their Premenstrual Mood Index. Ten belonged to the control group.

Aldosterone

There was a significant rise in plasma aldosterone in the premenstrual phase of all subjects to double the preovulatory levels ($p < 0{\cdot}005$). The aldosterone level of the symptomatic group was lower than that of the control group, although the difference did not achieve statistical significance (Fig. 1).

There was no significant correlation between Premenstrual Mood Index and the premenstrual aldosterone levels ($\rho = 0{\cdot}0830$).

Progesterone

Serum progesterone levels were similar in both groups at premenstrual, preovulatory and menstrual phases of the cycle. The level was significantly elevated in the symptomatic group during the postovulatory phase (Fig. 2). There was a weak but significant correlation between the Premenstrual Mood Index and the postovulatory progesterone levels ($\rho = 0{\cdot}4267$; $p < 0{\cdot}05$).

Spironolactone cycles

The administration of spironolactone significantly diminished the Premenstrual Mood Index in the symptomatic group ($p < 0{\cdot}005$; Table I). Figure 3 shows the values for each cycle of the trial. Two values represent subjects who took therapy for the first two days only; one commenced menstruation on day 20 and another discontinued therapy due to the onset of palpitations. Palpitations were recorded by two patients, the only reported side effect.

The mean weight showed only a slight increase (less than 1 kg) in the premenstrual phase. However, during spironolactone therapy the mean weight was reduced below preovulatory levels. This was found in both groups of subjects. Although the direction of change was consistent, it was small and did not reach statistical significance.

The administration of spironolactone caused a marked elevation of aldosterone in the premenstrual phase (Fig. 4) but did not significantly alter progesterone. Plasma aldosterone levels (mean ± SEM) were $14{\cdot}6 \pm 2{\cdot}3$ ng/100 ml (symptomatic) and $21{\cdot}5 \pm 4{\cdot}9$ ng/100 ml (asympto-

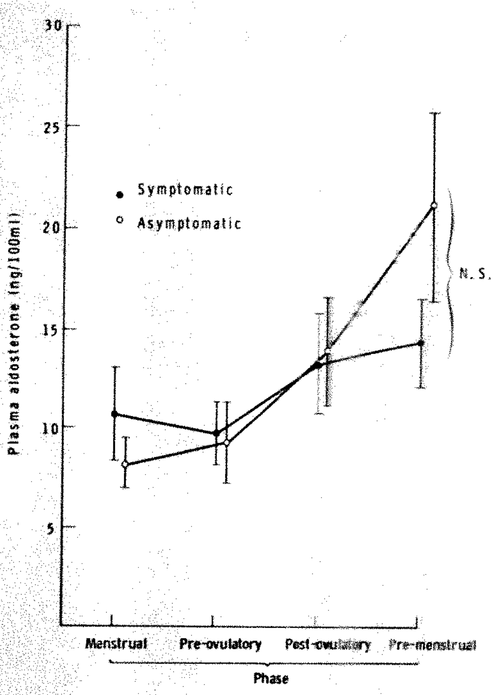

FIG. 1

Aldosterone levels (Mean ± SEM) for each phase of the menstrual cycle in symptomatic and asymptomatic subjects.

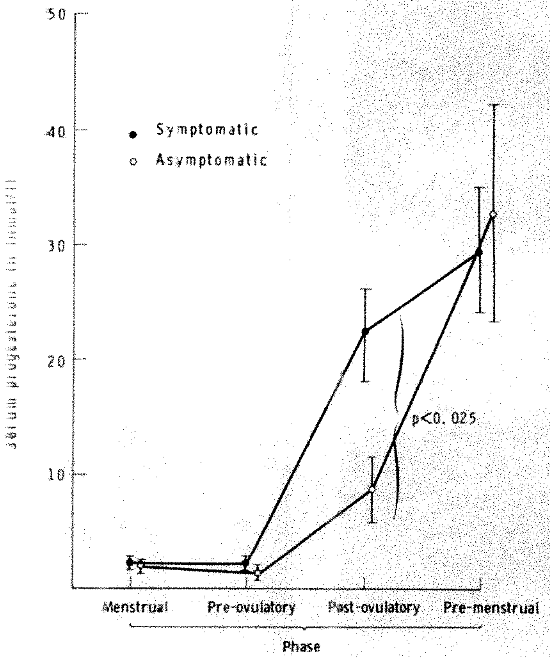

FIG. 2

Progesterone levels (Mean ± SEM) for each phase of the menstrual cycle in symptomatic and asymptomatic subjects.

matic) during untreated cycles. This increased to 29·5±4·7 ng/100 ml and 44·1±10·1 ng/100 ml during spironolactone therapy. Serum progesterone (mean±SEM) before treatment was 29·8±5·1 nmol/l (symptomatic group) and 32·8±9·4 nmol/l (asymptomatic) whilst during therapy levels were 27·1±3·8 nmol/l and 29·1±7·7 nmol/l respectively.

DISCUSSION

The administration of spironolactone during the luteal half of the menstrual cycle decreased the incidence of premenstrual mood symptoms (as indicated by the Premenstrual Mood Index) in more than 80 per cent of treated cycles, as compared with placebo cycles, although there was no evidence, to support the hypothesis that the aldosterone levels were higher in the symptomatic group. These results suggest that some

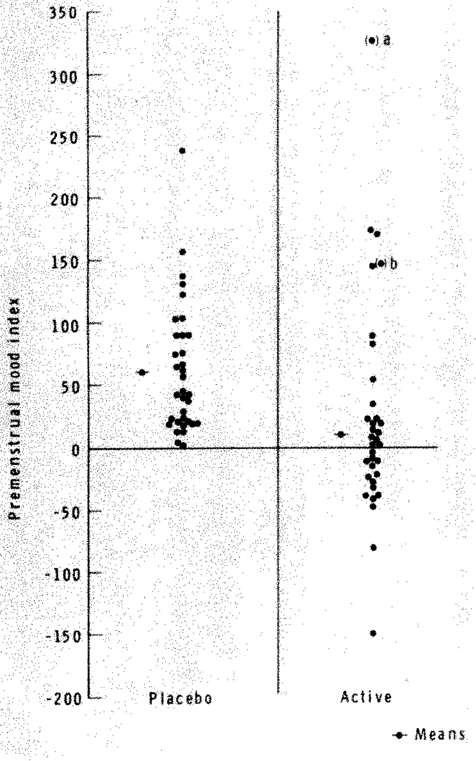

FIG. 3

Premenstrual Mood Index for the eighteen symptomatic subjects; placebo and treated cycles are shown. Two subjects took active treatment for only two days (a) began menstruating on day 20 and (b) stopped treatment because of palpitations.

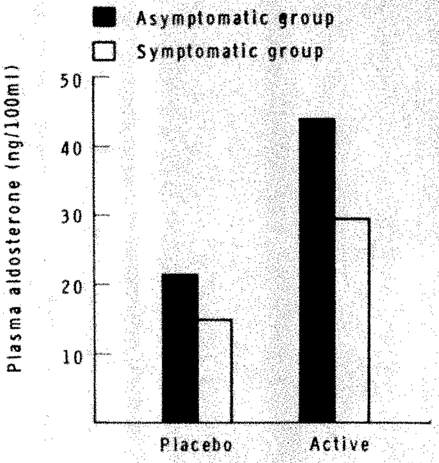

FIG. 4

Plasma aldosterone (Mean±SEM) levels in the premenstrual phase, showing the effect of spironolactone therapy in both groups.

TABLE I

Age, weight and Premenstrual Mood Index (PMI), showing the effect of spironolactone on weight and mood change

Patients	Age (years) Mean±SEM	Weight (kg) Mean±SEM Pre-ovulatory placebo	Weight (kg) Mean±SEM Pre-menstrual placebo	Weight (kg) Mean±SEM Pre-menstrual treated	Premenstrual Mood Index (PMI) Mean±SEM In both placebo months	Premenstrual Mood Index (PMI) Mean±SEM In both treated cycles	P value from Wilcoxon Test comparing the PMI in treated and untreated cycles
Asymptomatic (n = 10)	26±6	59·5±4·0	60·0±3·9	59·1±4·0	−14·0±12·0	22 ±18	p <0·5
Symptomatic (n = 18)	31±7	63·6±3·2	64·1±3·5	62·3±5·5	62·3±13·0	12·2±16	p <0·005

other factor is responsible for the change in fluid balance, with secondary suppression of aldosterone.

Weight increase in the premenstrual phase was not as great as expected, though it was slightly greater in the symptomatic group. The mean weight was reduced during spironolactone therapy to values below the preovulatory levels, suggesting a diuresis.

It is probable that spironolactone acted purely through its diuretic action. Previous studies of diuretic therapy in this syndrome have given varying results. Metolazone (Werch and Kane, 1976) relieved both physical and psychological symptoms when compared with placebo in women who were selected for weight increase of at least 1·35 kg. Relief of symptoms was similar to that found in the present study. Uncontrolled studies have shown that chlorthalidone (Kramer, 1962), chlorothiazide (Jungck *et al*, 1959) and quinethazone (Baden and Lizcano, 1963) relieved both types of symptoms. It is difficult to rule out a placebo effect which is well established in this syndrome. A single blind study showed chlorothiazide (Appleby, 1960) to be more effective than placebo. A double blind trial of chlorthalidone (Mattson and Schoultz, 1974) showed it to be no more effective than the placebo.

Spironolactone appears to be at least as effective as metolazone (the only diuretic previously shown to be effective in adequately controlled trials). Moreover, this improvement in symptoms occurred even in patients who were not selected on the basis of weight gain.

The long term use of spironolactone, cyclically, would avoid the possibility of secondary aldosteronism or the more severe cyclical oedema syndrome, which may result from inappropriate diuretic therapy.

Progesterone levels were significantly higher during the postovulatory phase in the symptomatic group; there was no difference in cycle length to account for this. This hormone has previously been shown to be natriuretic (Landau and Lugibihl, 1961), and the higher levels of progesterone found in this study cannot therefore account for symptoms by a direct renal mechanism. Indeed, previous workers have shown lower levels (Munday, 1977) and have suggested that the lower levels of this natriuretic hormone are responsible for the syndrome. The groups in those studies were not well-matched and their results are of doubtful significance. Progesterone has been administered by various routes (Dalton, 1977), and much success claimed, though there are no adequate double blind trials to support this. Progesterone may act to some extent by its diuretic action, though its placebo effect may be more significant.

Three possible theories remain from our findings: (i) Though there is no difference in aldosterone levels, individual sensitivity to its increase in the luteal phase may be different in the two groups and hence aldosterone may still be responsible.

(ii) It is possible that aldosterone is not responsible and spironolactone acts only as a diuretic. If that were so, then this would be the diuretic of choice since it would prevent the possibility of secondary aldosteronism or the more severe idiopathic oedema syndrome.

(iii) The most probable explanation is that the syndrome is of multiple hormonal aetiology and data will be available from further studies which should confirm or refute this.

ACKNOWLEDGEMENTS

The authors thank the volunteers in this study; Miss Jill Adcock and Mrs S. Holmes who performed the aldosterone and progesterone assays and Mr Roger Power for advice with the use of psychological data. We are also grateful to G. D. Searle for the supply of tablets and their financial support.

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GIANT CELL ARTERITIS OF THE FEMALE GENITAL TRACT

BY

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Summary

In a patient who had a hysterectomy and bilateral salpingo-oophorectomy for endometrial adenocarcinoma, a giant cell arteritis was found in many of the myometrial and tubal vessels and in a few of the small arteries of the cervix and ovaries. On subsequent questioning, a history of treatment for polymyalgia rheumatica during the preceding 12 months was elicited. Of the three previously reported patients with giant cell arteritis of the uterus, two were suffering from polymyalgia rheumatica whilst one possibly had disseminated visceral giant cell arteritis.

INVOLVEMENT of the female genital tract in giant cell arteritis is extremely rare; only three examples have been reported (Polasky *et al*, 1965; Pirozynski, 1976) and in each instance the vascular lesion was only found in the myometrium. We now report a patient with giant cell arteritis of the myometrium, cervix, Fallopian tubes and ovaries; later on we found that the patient had polymyalgia rheumatica.

CASE HISTORY

A 61-year-old widow presented with a history of postmenopausal vaginal bleeding for three months. No abnormality was detected on physical examination but diagnostic curettage produced copious material which proved to be a well differentiated adenocarcinoma of the endometrium. The patient was treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy and postoperative radiotherapy to the vaginal vault.

In view of the histological report on the operation specimen, the patient was specifically asked about muscle pains at the follow-up clinic. She now gave a history of having visited her family doctor over the previous 12 months with muscular pain in the pectoral and pelvic regions; aspirin had been prescribed with good symptomatic relief. Investigations now showed a normal blood count, an sedimentation rate of 65 mm in the first hour, an increase in alpha-2-globulin on plasma protein electrophoresis and a C-reactive protein level of 1 mg/dl; tests for rheumatoid factor and lupus erythematosos cells were negative.

Pathological report

The uterus was normal in size and shape but on sectioning the endometrial cavity was slightly dilated and contained at the fundus a mass of friable white tumour which appeared to be infiltrating only into the inner third of the myometrium; the uterus was otherwise normal as were the tubes and ovaries.

Microscopic examination confirmed that the tumour was a well differentiated invasive endometrial adenocarcinoma and also revealed a widespread inflammatory lesion of the small and medium sized arteries in the myometrium, cervix, tubes and ovaries. By no means all the vessels were affected and severely inflamed arteries and normal vessels were commonly seen side by side; the arteritis was most prominent and widespread in the myometrial and tubal vessels; only occasional arteries in the cervix or ovaries showed any abnormality. In the affected vessels the lumen tended to be narrowed, sometimes markedly so, by oedema and fibrosis of the intima whilst the brunt of the inflammatory lesion, which was granulomatous in nature, was borne by the media; often the granulomata were focal affecting a localized area of the media but in some vessels there was a circumferential replacement of the media by granulmatous tissue (Fig. 1). The granulomata (Fig. 2) were formed by histiocytes, epithelioid cells and giant cells which were usually of the Langhans type but occasionally of foreign body type. The adventitia was usually infiltrated by lymphocytes and there was no evidence of fibrinoid necrosis or infiltration of eosinophilic material in the wall of the inflamed vessels. A Ziehl-Neelsen stain for tubercle bacilli was negative but stains for elastic tissue (Fig. 3)

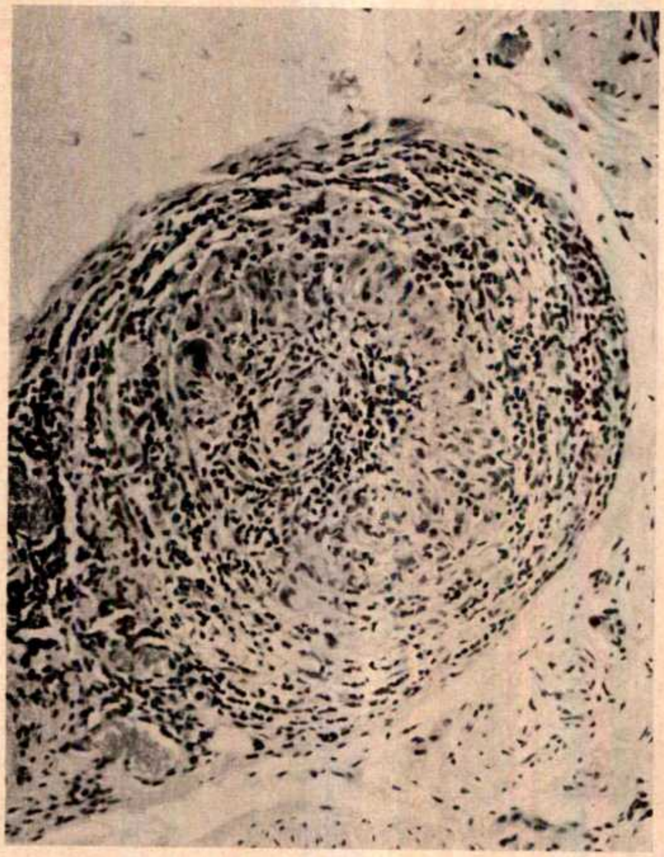

FIG. 1

A small artery in the Fallopian tube. Virtually all of the media is replaced by a granulomatous inflammatory infiltrate (haematoxylin and eosin ×75).

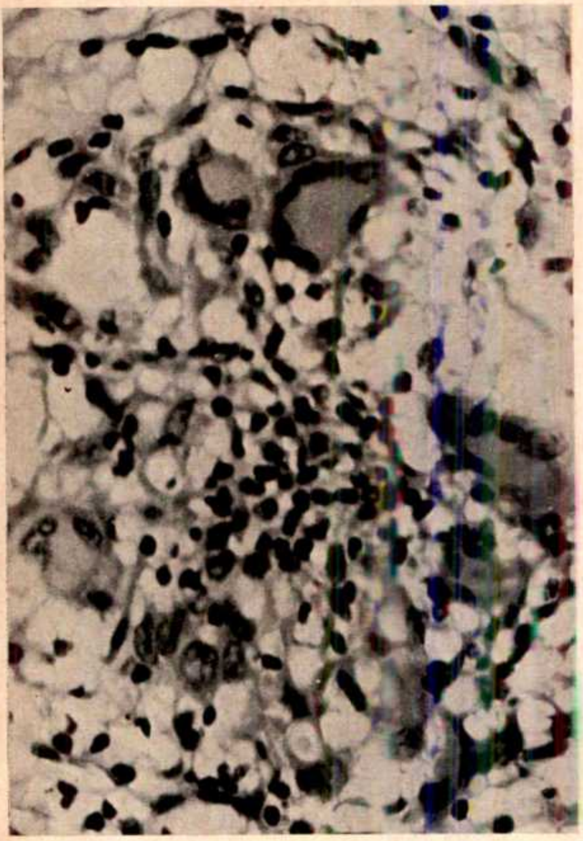

FIG. 2

High power view of a granuloma in a myometrial vessel; many giant cells of Langhans type are present (haematoxylin and eosin ×450).

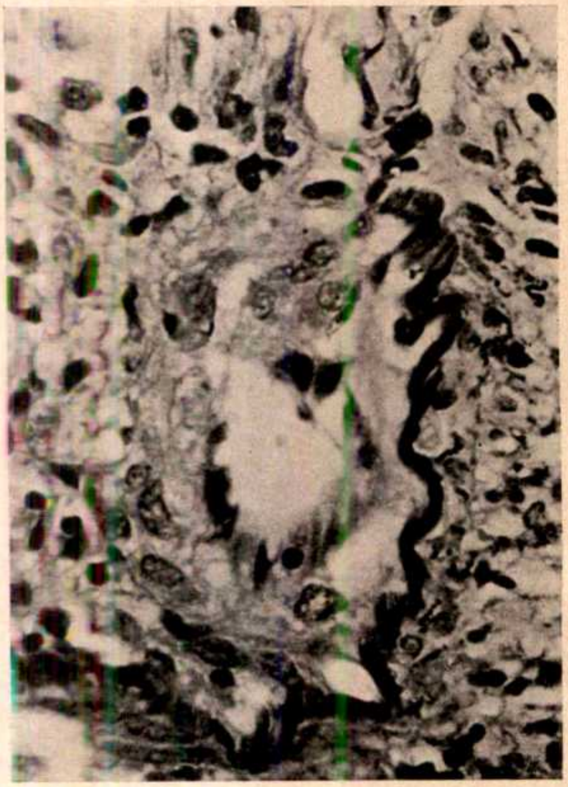

FIG. 3

A small artery in the Fallopian tube. The internal elastic lamina has been partially destroyed by a focal granulomatous lesion (elastic-van Gieson ×450).

showed destruction, thinning or fraying of the internal elastic lamina at those points where it was involved in the granulomatous lesions.

DISCUSSION

Giant cell arteritis most commonly involves the temporal artery and whilst the aorta and its main branches can also be affected (Cooke *et al*, 1946; Hepstinstall *et al*, 1954; Ostberg, 1972; Klein *et al*, 1975) the only site outside the female genital tract in which small artery involvement has been noted is the breast (Waugh, 1950).

The pathogenesis of giant cell arteritis is not known but it has gradually become clear that there is a very close relationship between this form of arteritis and polymyalgia rheumatica; this latter condition is characterized by pain and stiffness in proximal muscle groups, particularly those of the pelvic and pectoral girdles, and not only do many patients with clinically diagnosable temporal arteritis have, or develop, polymyalgia rheumatica but temporal artery biopsies in patients with polymyalgia rhematica usually reveal clinically silent arterial disease (Alestig and Barr, 1963; Bruk, 1967; Wilske and Healey, 1967). In our patient the pathological findings prompted further questioning which uncovered a typical history of polymyalgia rheumatica; of the three previously reported patients with giant cell arteritis of the uterus, two were also considered to be suffering from polymyalgia rheumatica. One had no evidence of this disease (Pirozynski, 1976) but may instead have had disseminated visceral giant cell arteritis (Lie, 1978); this condition affects extracranial small arteries and differs from the classical form of giant cell arteritis in the lack of involvement of the temporal artery and the absence of any association with polymyalgia rheumatica. The histological findings in disseminated visceral giant cell arteritis do not, however, appear to differ significantly from those of the classical form of giant cell arteritis and hence distinction between the two conditions can probably not be made on histological grounds if giant cell arteritis is found in the small vessels of an extracranial organ.

Giant cell arteritis of the uterus, though apparently severe, does not appear to lead to any local symptoms.

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FAMILIAL CARCINOMA OF THE OVARY
CASE REPORT

BY

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Summary

A patient was successfully treated by surgery, progestogens and chlorambucil for a poorly differentiated cystadenocarcinoma of the ovary. Four of her relatives had died of ovarian cancer; it was ascertained that two of them also had poorly differentiated cystadenocarcinoma.

CASE REPORT

IN 1973 a patient aged 53 who had had yearly pelvic examinations was found to have a large abdominal tumour which caused her frequency of micturition and stress incontinence.

A laparotomy was done on 28th June, 1973. A large obviously malignant tumour was found filling the pelvis, with the uterus pulled up and straddling the front of the tumour. The tumour was adherent to the large bowel posteriorly. The bladder was pulled up high over the anterior surface of the uterus and the tumour. No metastasis was found anywhere in the abdomen and the liver was normal. During the operation the bladder was pushed down and the ureters were identified. The uterus, tubes, both ovaries and a small cuff of the vagina were removed. After this a small fragment of tissue was found on the posterior wall of the peritoneum just below the sacral promontory. It was removed as was a knuckle of large bowel which had been adherent to the tumour. Convalescence was rapid and uneventful, but the patient had retention of urine and had to be catheterized for three days. The patient left hospital on the ninth postoperative day.

The late Dr Michael Peters reported that the right ovary which measured 14 × 8·5 × 7 cm showed rather poorly differentiated and pleomorphic serous cystadenocarcinoma, haemorrhagic and necrotic in places. Mitoses were numerous but neither lymphatic nor venule involvement was found. A secondary growth in the peritoneum was confirmed and a few loose neoplastic cells were seen in the exudate over the serosa of the large bowel.

The patient started pelvic cobalt teletherapy on 15th July, 1973 and received a total dose of 5365 rads (Dr D. B. L. Skeggs).

On 27th September, 1973 cystoscopy and examination under anaesthetic revealed extensive infiltration of the left side of the bladder with growth reaching out to the side wall of the pelvis. The patient was given 100 mg of medroxyprogesterone acetate (Provera) three times daily together with chlorambucil 10 mg daily for 14 days. The patient is still on medroxyprogesterone acetate but the chlorambucil was discontinued after 3½ years, having been given in pulses of 10 mg daily for 14 days with intervals of 21 days between treatments.

Information obtained from medical members of the family in Canada and South Africa revealed that out of 10 women at risk in two generations of the family, five (including the patient reported here) had developed ovarian carcinoma, and it was possible for Dr Peters to confirm histologically the similarity of material from two of the other patients with that obtained from mine. The other four women had all died of the disease and were the patient's mother (one of three sisters and three brothers), her mother's sister, that sister's daughter (one of four boys and three girls) and another maternal aunt's daughter (one of three sisters). All remaining female first cousins have now had a prophylactic hysterectomy and bilateral salpingo-oophorectomy.

DISCUSSION

The occurrence of familial breast cancer has been reported frequently and causes postulated include genetic abnormality, hereditary lack of immunity to transmission of Bittner's milk factor and infection with unidentified viruses.

As for familial ovarian cancer, Liber (1950) reported a family with malignant ovarian adenocarcinoma in a mother and five daughters; he was apparently the first to recommend prophylactic bilateral salpingo-oophorectomy.

Feld *et al* (1966) described bilateral ovarian dermoid cysts in triplets. Lewis and Clare Davison (1969) reported a mother and five daughters who died of ovarian carcinoma. Molloy (1970) reported two sisters who died of anaplastic ovarian carcinoma and Lynch and Krush (1971) reported a combination of cancer of the breast and of the ovary in three families; and Lynch *et al* (1974) discussed this combination. There are other reports of familial ovarian cancer (McCrann *et al*, 1974; Fraumeni *et al*, 1975).

The papers of Tortora (1967), Serr *et al* (1969), Fraumeni *et al* (1975) and Lingeman (1974), who gives 157 references, are all worth perusing before embarking on further research.

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A REVIEW OF IMMUNOLOGICAL ASPECTS OF GYNAECOLOGICAL MALIGNANCY

BY

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THE clinical applications of tumour immunology may be considered under three headings—immunodiagnosis, immunocompetence and immunotherapy. Some aspects of all three are of particular interest in gynaecological cancer.

IMMUNODIAGNOSIS

Immunodiagnosis is a new field of investigative medicine largely based on the science of radio-immunoassay. Substances which are antigenic in animals, or which can be bound to antigens, can be measured in body fluids in very low concentrations. Tumour immunodiagnosis depends on the liberation by tumours of such substances into the blood stream or other body fluids in a form or concentration not commonly found in healthy individuals. These substances, usually called tumour markers are sometimes referred to as antigens though they do not necessarily produce an immune response in the tumour bearing host. Some of these substances may actually play a role in protecting tumour cells from the host's immune response.

Five groups of tumour markers may be recognised: (i) hormones (appropriate or inappropriate); (ii) oncofetal antigens; (iii) pregnancy associated plasma proteins (PAPP); (iv) acute phase proteins; (v) tumour associated antigens (TAA).

Hormones

Hormone production in gynaecological cancer is well recognised and the value of a tumour marker is demonstrated by the role of human chorionic gonadotrophin (HCG) assay in the diagnosis and treatment of invasive trophoblastic tumours (Bagshawe, 1977). Because the concentration is a direct reflection of tumour mass the assay can be used to titrate chemotherapy.

Inappropriate hormone production is occasionally responsible for clinical endocrine syndromes in malignant disease. The best known example is Cushing's syndrome which most commonly occurs as a result of ectopic adrenocorticotropic hormone (ACTH) production by bronchial carcinoma. There is little evidence of this in gynaecological cancer (Nichols *et al*, 1962). The serum of a series of ovarian cancer patients has been screened for excess ACTH with only one marginally positive result (Dr Lesley Rees, personal communication). Disturbances of calcium metabolism of an endocrine nature do occur (Rees, 1975) and I have seen a patient with very advanced carcinoma of the vulva with hypercalcaemia not apparently due to secondary hyperparathyroidism; there is also a recognised association between 'clear cell' carcinoma of the ovary and hypercalcaemia (Ross and Shelly, 1968).

Hormones may also be produced by tissue not normally found in the organ which is the site of neoplasm. An example of this is the production of thyroxine by a struma ovarii or the production of 5-hydroxytryptamine (5HT) by a carcinoid tumour. (Robboy *et al*, 1974).

Oncofetal antigens, pregnancy associated plasma proteins, and acute phase proteins

Ectopic trophoblastic tissue in germ cell tumours is responsible for increased plasma levels of HCG, placental lactogen and pregnancy specific glycoproteins. The recognition of trophoblastic tissue in such tumours is of great importance as it always implies malignancy (Wider *et al*, 1969). More recently small amounts of the pregnancy associated proteins have been found in a wide range of tumours including those of breast and bowel (Horne *et al*, 1976). Raised serum levels of the β sub-unit of HCG have been found in some 30 per cent of patients with primary ovarian carcinoma (Stone *et al*, 1977).

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Germ cell tumours in either sex, including those of extra-gonadal origin, may show other extra-embryonic differentiation towards yolk sac tissue. The 'endodermal sinus tumour' of adolescence and young adult life commonly produces alpha-fetoprotein (Abelev, 1974). Until the advent of chemotherapy, this tumour, which must be distinguished from mesonephroma, ran a uniformly fatal course (Smith, 1975).

Alpha-fetoprotein is regarded as an onco-fetal antigen because it is present in fetal life and embryonal tumours of yolk sac origin and also in adult hepato-cellular carcinoma. It is not normally found with common epithelial tumours of the ovary (Levin *et al*, 1976*a*).

Other onco-fetal antigens are recognised, of which carcinoembryonic antigen (CEA) is the best known and still probably the most useful non-specific tumour indicator substance available (Gold and Freedman, 1965). CEA is found with bowel cancer and in some cancers from other sites including ovary and uterus (Barralet and Mach, 1975). The occasional elevation of CEA in some benign inflammatory conditions means that it has little practical value as a non-specific screening test for malignancy. Once primary malignancy has been diagnosed and treated, however, CEA has a definite role in follow up and surveillance (Khoo and Mackay, 1976).

Attempts have been made to identify enzymes and other protein substances liberated by malignant tissues. C-Reactive protein and enzymes such as lactic dehydrogenase (Awais, 1973) are in this category. Many of the latter merely reflect tissue damage and necrosis and are the so-called acute phase proteins. Their value in the management of malignant disease is strictly limited, as the time interval between the appearance of elevated levels and clinically overt disease is rather short (Coombes *et al*, 1977).

Tumour associated antigens (TAA)

Finally, in the list of possible tumour markers come tumour associated antigens (TAA). In the human, evidence for the liberation of specific neo-antigens is very scanty, but whatever their nature and specificity, TAA and the corresponding autologous antibodies are theoretically capable of measurement. In ovarian cancer, earlier claims for specificity of tumour extract antigenicity have not been substantiated (Levi, 1971; Barber *et al*, 1975). In ovarian cancer, the host immune response is clearly complex as the lymphocytes from some patients with ovarian cancer will react to fetal ovary extract (but not fetal lung or liver) and to a lesser extent extracts of normal adult ovary, and there is some cross reaction with other cancers (Levin *et al*, 1975). Fractionation suggests that the major activity is in the 15 000 daltons region (T. Poulton, unpublished observation). There is no satisfactory diagnostic aid yet developed in this area, but the potential exists.

IMMUNOCOMPETENCE

With immunocompetence, it is necessary to consider ways in which the host response against tumours may be suppressed and whether or not there is evidence in the human that a tumour directed response occurs. Circumstantial evidence for a tumour directed host response comes from the rare instances of spontaneous regression of secondary deposits after removal of the primary, and from patients in whom there is a prolonged interval before the appearance of metastases. Gynaecological cancer, particularly of the ovary, features regularly in accounts of the above phenomena. Evidence for a cell mediated immune response in ovarian cancer has been claimed by various workers, using different laboratory techniques (R. L. Powles, personal communication; Levin *et al*, 1976*b*; Di Saia, 1975) and with limited specificity in carcinoma of the cervix (Singer *et al*, 1975; Sharp *et al*, 1977, personal communication). This response is not generally detectable when the disease process is active, and the nature of the antigenic stimulus is uncertain. The response may be evoked by transplantation allo-antigens, intracellular antigens liberated by tissue necrosis (Whitehouse and Holborow, 1971) bacterial antigens (Alexander, personal communication), ferritin subunits (Marcus and Zinberg, 1974) or Regan alkaline phosphatase (Stolbach *et al*, 1969).

It has been shown by Hellstrom *et al* (1968) and others that the serum of patients with active cancer is capable of blocking *in vitro* evidence of a tumour directed cell mediated immune response. This response may sometimes be unblocked *in vitro* by careful washing or pre-incubation of lymphocytes but blocking in advanced disease probably accounts for some failures to demonstrate a cell mediated response (Chatterjee *et al*, 1975). The mechanism of blocking is not clearly understood, but an excess of antigen or the formation of immune complexes are two possibilities (Poulton *et al*, 1978). The blocking phenomenon may reflect a loss of natural resistance to malignant disease. Possibly surface sialomucins and pregnancy proteins have an additional protective effect on the cancer cell.

The immune status of cancer patients is another matter of clinical importance. General incompetence of the immune system may be congenital or acquired and is of particular importance when iatrogenic. General immunosuppression is a feature of terminal malignant disease but this is not the cause of the blocking action just mentioned, which precedes general immunodepression. Patients on immunosupressive therapy are more prone to malignant disease, sometimes of a rare and bizarre nature (Hoover and Fraumeni, 1973). Patients with Hodgkins disease treated by combination chemotherapy may show a similar phenomenon (Canellos *et al*, 1975) and there is a reported leukaemia risk after similar treatment of ovarian cancer (Reimer *et al*, 1977). Chronic immunosuppression may lead to increased susceptibility to virus infections such as the herpes zoster and herpes simplex are implicated in the aetiology of cervical cancer.

The role of chemotherapy in operable and inoperable breast and ovarian cancer is under investigation (Bonnadonna *et al*, 1976, MRC Study).

IMMUNOTHERAPY

If there is an immunological host reaction to malignant tumour formation, stimulation or initiation of this immune response might confer benefit. Immunotherapy may be active or passive, and specific or non-specific. Passive immunotherapy may be cell mediated or humoral.

Xenogeneic lymphocytes from a pig sensitized to tumour extract have been used in terminal cancer patients (Syme and Riddell, 1973). Passive humoral immunotherapy consists of the administration of specific antisera. Unfortunately such treatment presents distinct hazards. The formation of antigen/antibody complexes may actually promote the blocking effect mentioned earlier and tumour progress may be enhanced by certain antibodies (Chard *et al*, 1967). One aspect of passive immunotherapy which is currently receiving attention is the bonding of a cytotoxic agent to an antibody raised against the tumour antigen. This interesting idea awaits evaluation (Davis *et al*, 1978; Newman *et al*, 1977).

Active immunotherapy has been more widely studied and Graham and Graham (1962) reported experimental work in gynaecological cancer. Specific immunotherapy consists of provoking a cell mediated response to specific autologous or allogeneic tumour extract. There is a risk of enhancement by the inadvertent stimulation of a humoral response, but this risk is minimal with the intradermal injection of irradiated cryopreserved cells. This regimen has been used in acute leukaemia (Mathé, 1971; Powles *et al*, 1973) and malignant melanoma (Currie and McElwain, 1975). A small uncontrolled study in ovarian cancer has also been the subject of a preliminary communication (Hudson *et al*, 1976). There are few reliable prospectively randomized data on the value of active specific immunotherapy in solid tumour malignancy and these have tended not to support initial enthusiasm based on uncontrolled studies (Newlands *et al*, 1976).

Immunotherapy is unlikely to be more than adjuvant to conventional methods of treating cancer. But it may help to eradicate minimal residual disease. It is important that the immunogenicity of any tumour 'vaccine' should be verified experimentally before clinical studies of immunotherapy are undertaken.

Active immunotherapy may also be non-specific, BCG, *Corynebacterium parvum* and the drug levamisole being popular immunostimulatory agents. Many reports are anecdotal but the results of a prospective controlled study of intraserous BCG in bronchial carcinoma appear promising (McNeally *et al*, 1976). The dosage appears critical and BCG overdosage can be fatal (Sparks *et al*, 1973). In the gynaecological field these measures are experimental, but attempts have been made to exploit HLA differences in choriocarcinoma, where at least part of the tumour genome is of parental origin (Bagshawe, 1968). Topical application of a non-specific mitogen dinitrochlorobenzene (DNCB) has been used in carcinoma *in situ* but a satisfactory prospective evaluation is awaited (Guthrie and Way, 1975).

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BOOK REVIEWS

Atlas of Gray Scale Ultrasonography. By KENNETH J. W. TAYLOR. Pp 411, with numerous illustrations. Churchill Livingstone, Edinburgh, 1978. Price: £19.50.

THE introductory chapters provide a simple clearly written summary of the basic principles and practical aspects of diagnostic ultrasound. Thereafter the liver and pancreas are dealt with in considerable detail and for the uninitiated the pathological features are preceded by a short valuable section which covers the ultrasonic features of normal anatomy. The spleen and kidney are similarly covered followed by a short section on the great vessels. The section on obstetrics starts with an all too brief summary of the indications for ultrasonic examination of the pregnant uterus. It is a pity that in this introduction the author still uses the word maturity when he means gestational age and also omits to mention that fetal heart movement can be detected from approximately seven weeks menstrual age—vital to the management of threatened abortions. Although an example of anencephaly is shown there is no mention of the ultrasonic diagnosis of spina bifida. The gynaecological chapter again starts with a display of normal anatomy before covering some of the more readily identifiable pathological states. A short section on the thyroid follows and the atlas is concluded by a chapter demonstrating the very limited value of ultrasonic exmination of the lungs. The quality of picture reproduction is good and although the whole abdomen is adequately covered the obstetrician will be disappointed to find that the techniques of liver and renal biopsy are discussed whereas that of amniocentesis is inexplicably omitted. As an atlas it has a great deal to commend it to those who provide a non-obstetric service and even the ultrasound department in large obstetric units would be well advised to have a copy in the departmental library. The publishers are to be congratulated on keeping the price below £20—a feat not often achieved today with this quality of book.

M. J. Bennett

Advances in Obstetrics and Gynecology. Edited by R. M. CAPLAN and W. J. SWEENEY, III. Pp xv+710, with figures and tables. The Williams & Wilkins Company, Baltimore, 1978. Price: $49.50.

FROM the preface to the final chapter on the immunology of gynaecological cancer this is a book of solid worth. The editors, from Cornell University, have stretched beyond North America for contributors, many of whom are of the highest international standing. Thus, for example, Bagshawe deals with trophoblastic disease, Csapo with monitored induced labour, Freedman with patterns of labour, Howard Jones with congenital anomalies, Kistner with the menopause and Shearman with induction of ovulation; but Donald writes on high fetal risk pregnancy, with the use of diagnostic ultrasound in obstetrics discussed by Gottesfeld and colleagues from Colorado. The chapters, 26 obstetrical and 28 gynaecological, are short, averaging 13 pages each but they are succinct and reasonably well referenced. The limited extent of some recent advances, e.g. in the immunology of pregnancy, is freely admitted. Nine of the chapters are concerned with aspects of gynaecological cancer, but the spread of subjects is wide enough to include carcinoma of the breast in pregnancy and lactation, thyroid and adrenal problems in gynaecology and osteoporosis in the female. The editors are concerned with perspective and priorities. One paragraph in the preface reads 'Beyond specific scientific advances, it is important for every physician to realise that he practices within the context of the mores and resources of the society in which he lives. Although his primary responsibility must always be to the individual patient, he must use the resources at his disposal as efficiently as possible'.

S. Bender

Neurology of Pregnancy. Vol. 7 in the Series Major Problems in Neurology. By J. O. DONALDSON. Pp xiv+271, with figures and tables. W. B. Saunders Company, Philadelphia, P.A., 1978.

THIS monograph is another excellent review from the series entitled 'Major Problems in Neurology' and it upholds the high standard of the previous volumes. Dr Donaldson's enthusiasm shines through in this comprehensive text, which discusses neurological problems approached from opposite viewpoints by neurologists and obstetricians, who may both sometimes, therefore, neglect the middle ground. The problems range widely from the commoner complications of the oral contraceptive drugs and problems concerning the drug treatment of epilepsy, to the rarer problems of management of the myasthenic mother and child. Some topics discussed, such as the control of delivery in paraplegics, though very rare indeed, require special management and are not neglected. Dr Donaldson has an historical bent and includes some rather horrific details of early neurological-obstetric complications which add spice to the text. There are comments on recently described syndromes such as the prolactinoma, and it is good to see a clear warning of the dangers of visual field encroachment when such a tumour increases in size if pregnancy is artificially induced. With the increasing frequency of epidural anaesthesia in labour, this might be thought to warrant a fuller discussion. In commenting on the use of intravenous hydralazine for severe hypertension, a word might be added concerning the dangers of reducing the blood pressure too swiftly. Attention is also given to the major, more specifically neurological problems of multiple sclerosis, cerebro-vascular disease and infections, as well as topics which are of more recherché neurological interest. The value of this text, which will be appreciated equally by neurologists and obstetricians, is enhanced by very careful bibliographies at the end of each chapter, which could not easily be amassed from any other source.

Sir Roger Bannister

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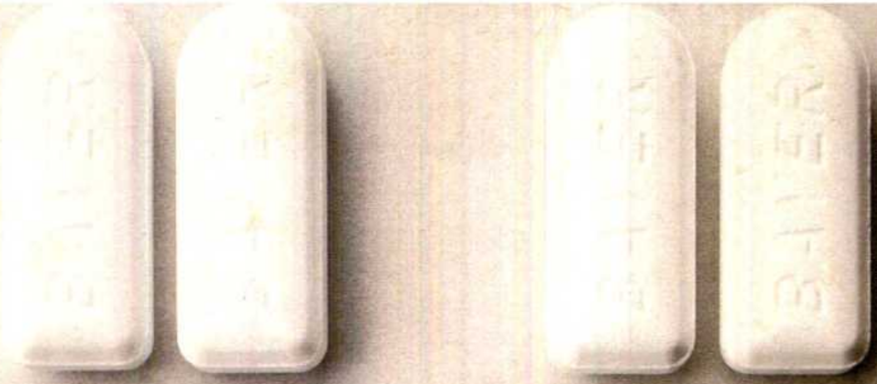

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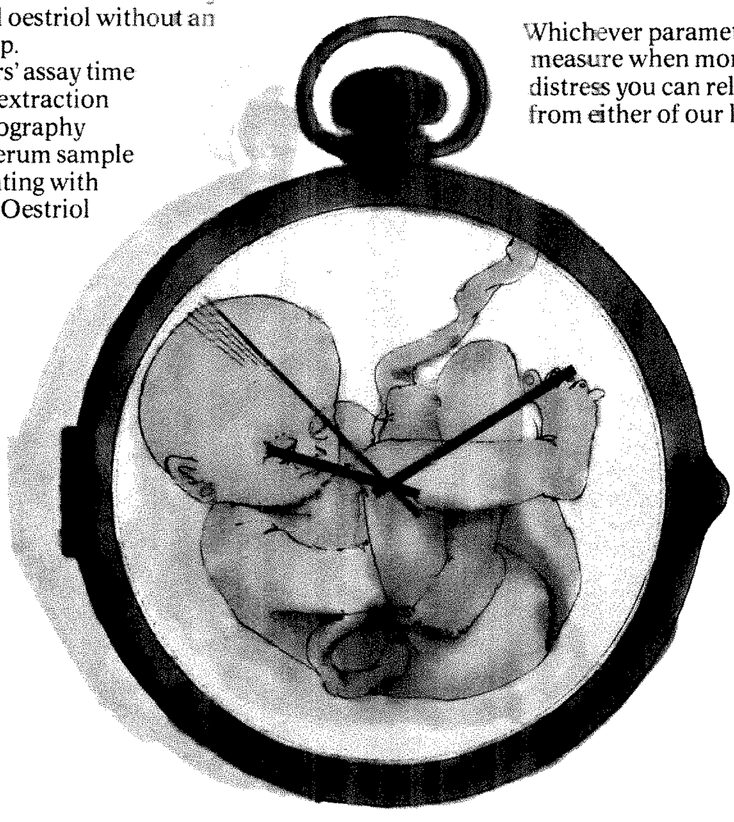

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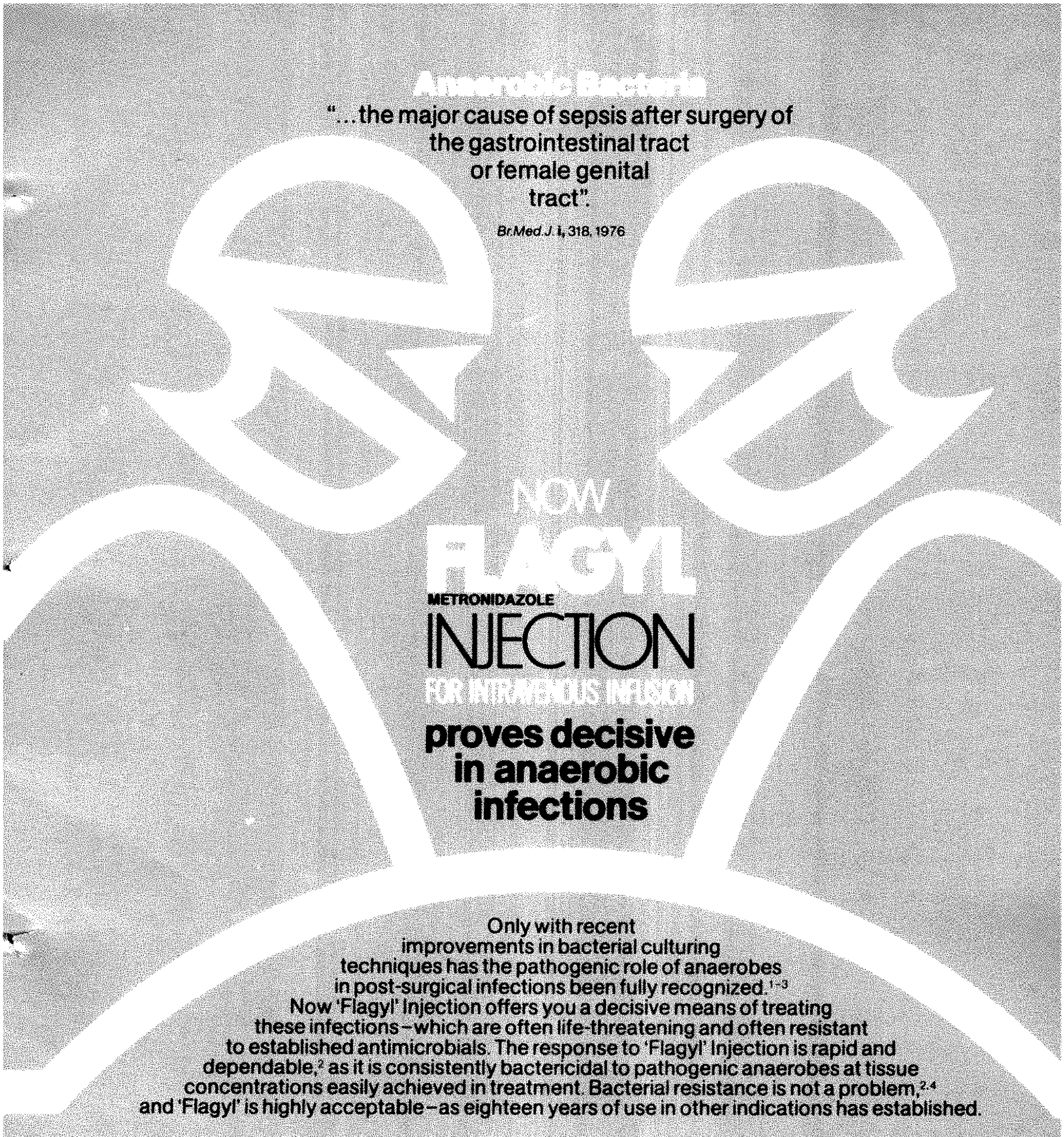

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"...the major cause of sepsis after surgery of
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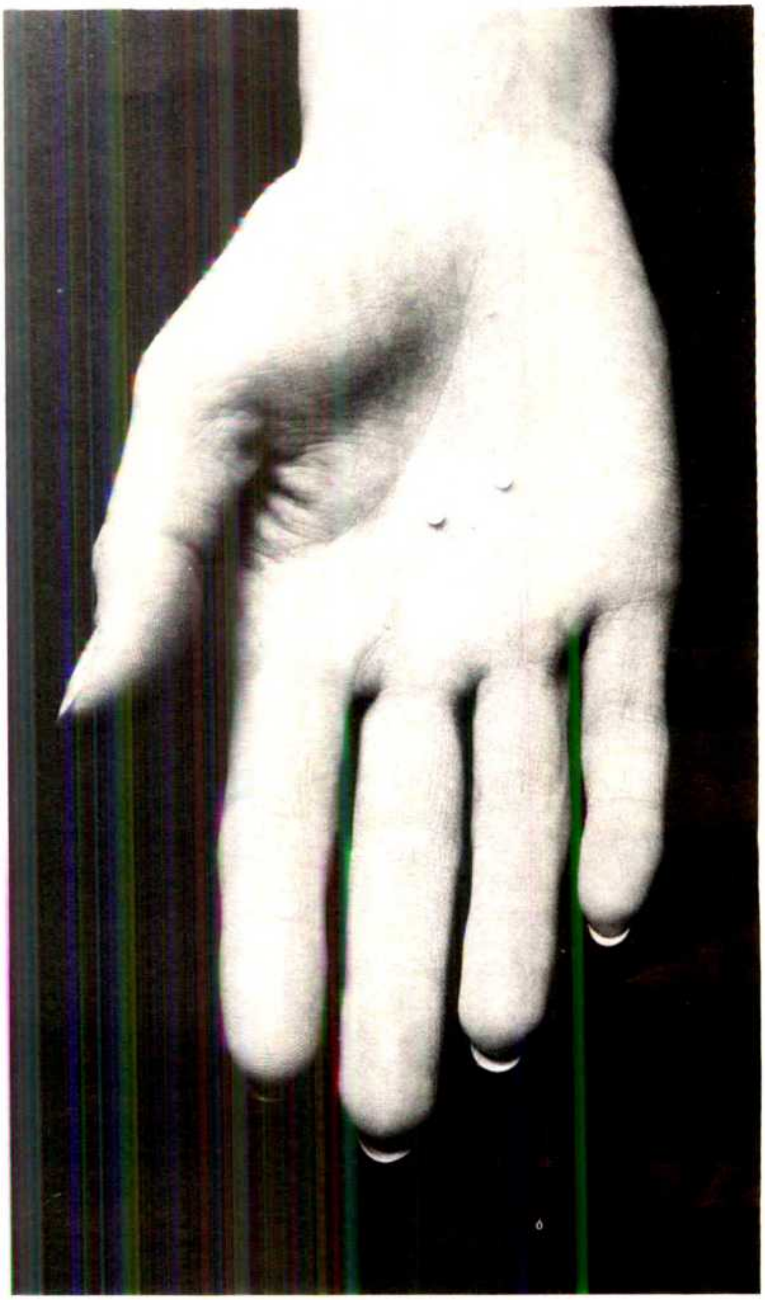

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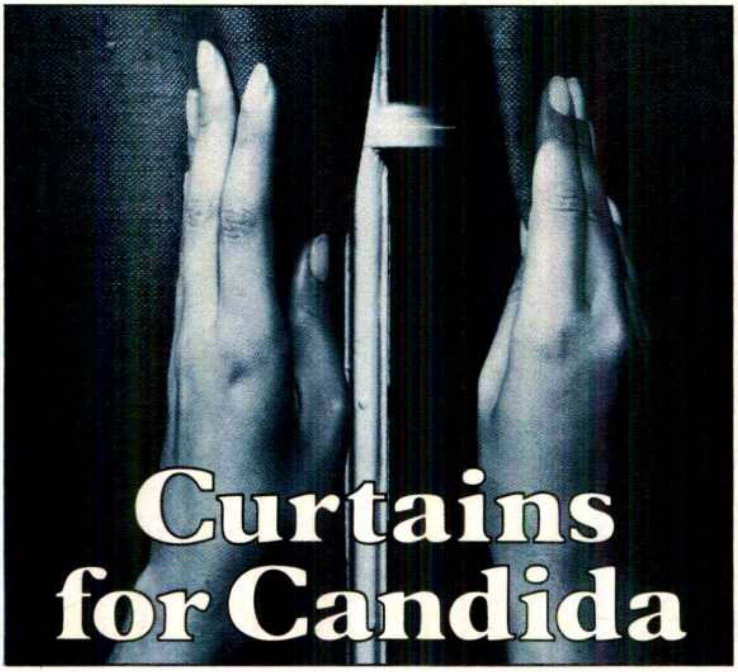

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USES

Actions Econazole nitrate has a broad spectrum of antifungal activity. It is highly active against Candida albicans and other Candida species and is effective in controlling infections of the vagina and vulva caused by such organisms (thrush).

Indications Vulvovaginal candidosis.

DOSAGE AND ADMINISTRATION

One pessary to be inserted at bedtime for 3 consecutive nights.
Administration should be continued even if menstruation occurs, and despite the disappearance of signs and symptoms of the infection.
The pessary should be inserted high into the vagina while the patient is supine. Although a three day course of therapy usually suffices it may be necessary to institute a second course of therapy.

CONTRA-INDICATIONS, WARNINGS, etc.

Contra-indications None known.

Precautions Ecostatin pessaries are effective in the candidal vaginitis associated with pregnancy. However, as with other agents, it should not be used during the first trimester of pregnancy unless the physician deems it's use essential for the welfare of the patient.

Side Effects Very occasionally patients may complain of discomfort; this is usually transitory and disappears with continued treatment. Rarely is it necessary to discontinue econazole pessary treatment.

PHARMACEUTICAL PRECAUTIONS

Storage Store at room temperature.

LEGAL CATEGORY POM

PACKAGE QUANTITIES Packs of three pessaries with applicator.

PRODUCT LICENCE NUMBER, NAMES AND ADDRESSES

Product Licence Number 0085/0013.

References

1. Avram, A. et Grupper, Ch. Bulletin de la Société Française de Mycologie Médicale 1975 Tome IV (2).
2. Preusser, H.J. et al. Research Report 1974. On file Cilag-Chemie.
3. Schweisfurth, R. Research Report 1974. On file Cilag-Chemie.

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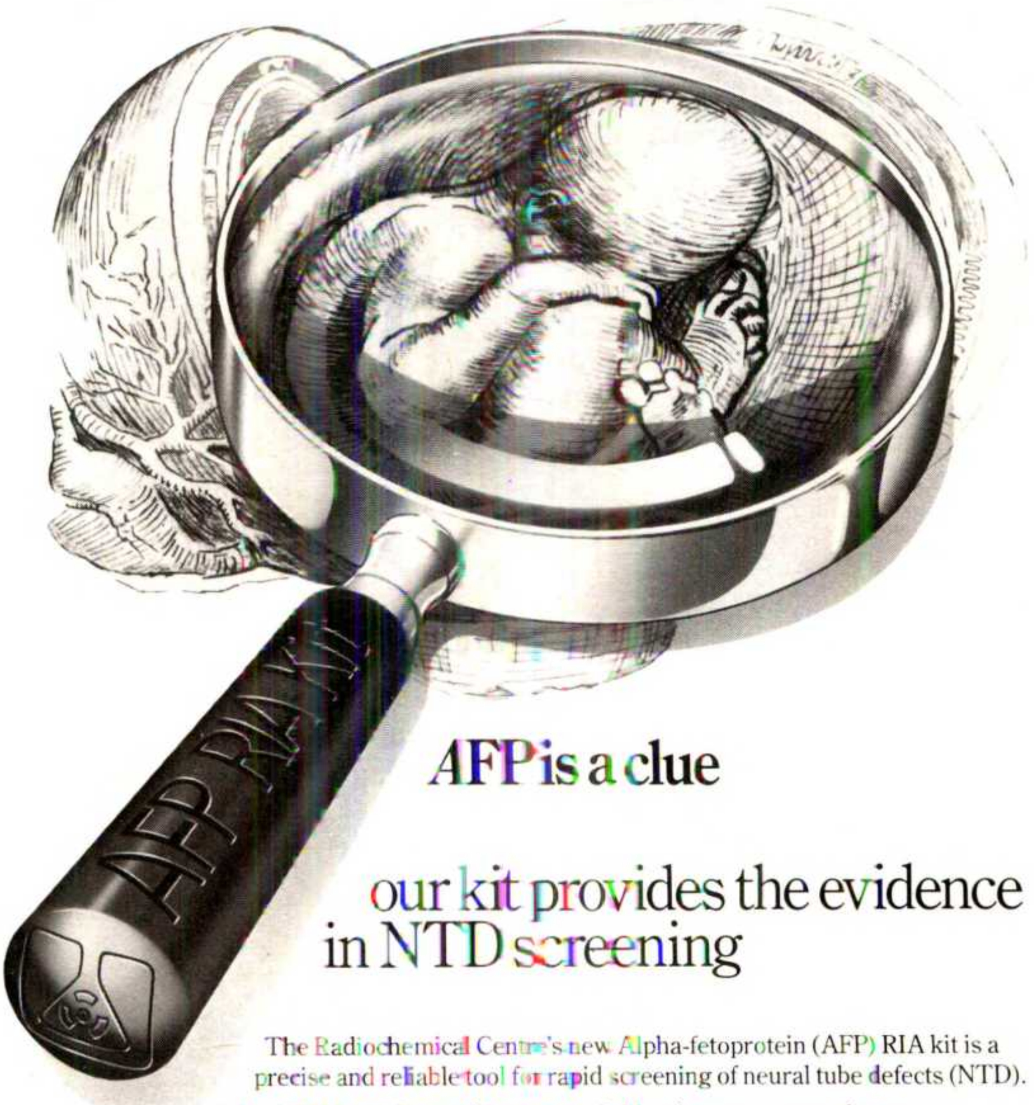

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Contra-indications, Warnings etc.
Contra-indications: None known.
Side-effects: None known.
Overdosage: Not applicable.
Precautions: Hypersensitivity has never been recorded, but if it should occur, administration of the product should be discontinued.
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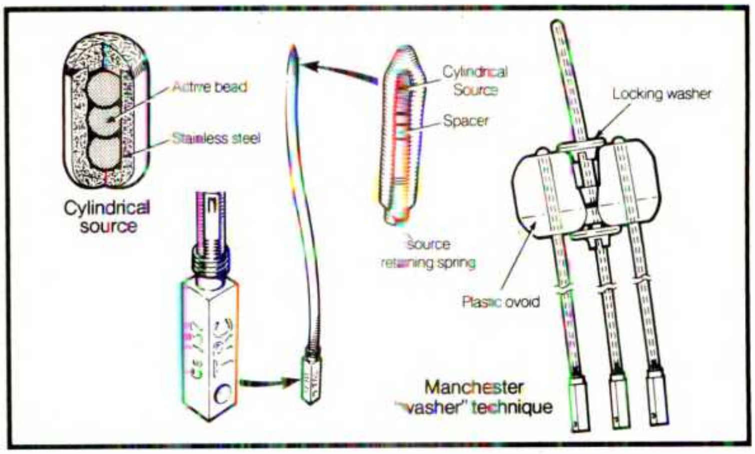

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Cefoxitin sodium, MSD

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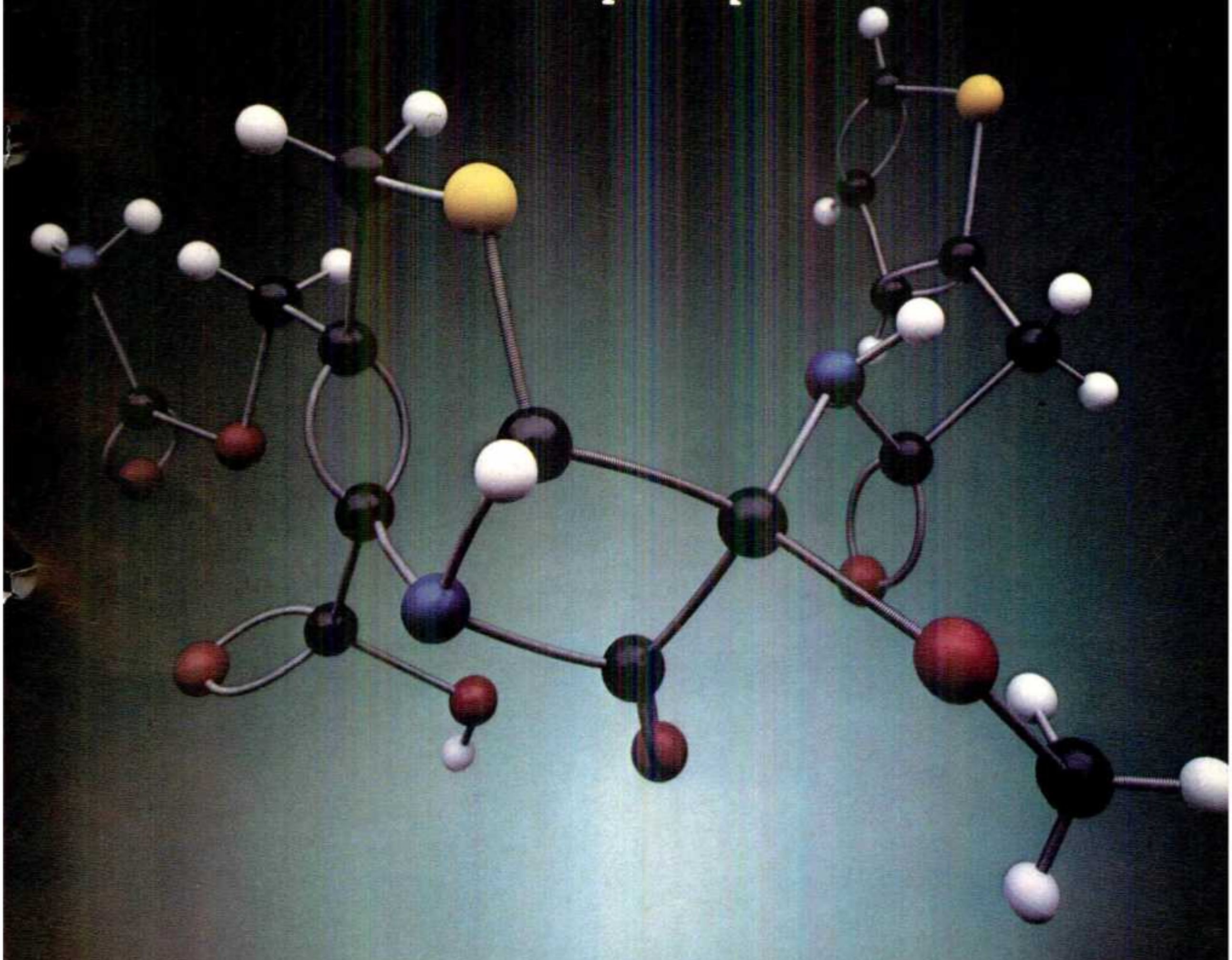

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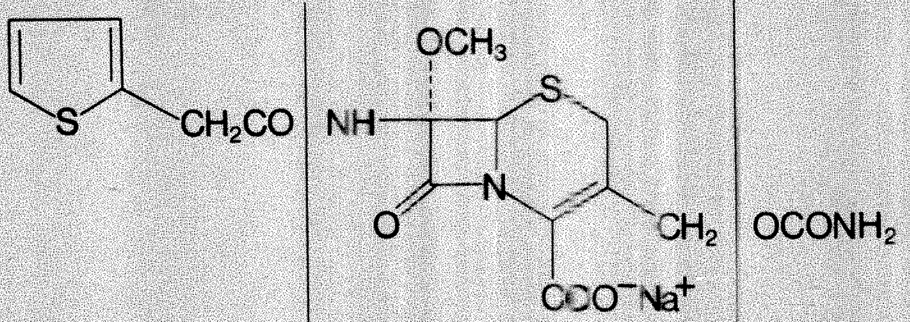

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'Mefoxin' is active *in vitro* against:

Aerobic bacteria

Gram-positive cocci including: Staphylococci (including coagulase-positive, coagulase-negative, and penicillinase-producing strains), Group A beta-haemolytic streptococci *(Streptococcus pyogenes)*, Group B beta-haemolytic streptococci *(Streptococcus agalactiae)*, *Streptococcus pneumoniae (Diplococcus pneumoniae)*, other streptococci (except group D streptococci including enterococci, most strains of which are resistant, e.g. *Streptococcus faecalis). Gram-negative cocci including: Neisseria gonorrhoeae* (including penicillinase-producing strains), *Neisseria meningitidis*. *Gram-negative rods, facultative anaerobes* including *Escherichia coli, Klebsiella pneumoniae*, Klebsiella spp., *Proteus mirabilis*, *Proteus* (indole-positive): *Proteus vulgaris, Proteus rettgeri, Proteus morganii, Haemophilus influenzae, Serratia marcescens*, Providencia spp., Salmonella and Shigella spp.

Anaerobic bacteria

Gram-positive cocci including: Peptococcus spp., Peptostreptococcus spp., Micro-aerophilic streptococcus. *Gram-positive rods including: Clostridium perfringens*, Clostridium spp., Eubacterium spp., *Propionibacterium acnes*. *Gram-negative cocci including:* Veillonella spp. *Gram-negative rods including: Bacteroides fragilis, Bacteroides melaninogenicus*, Bacteroides spp. (including both penicillin-susceptible and penicillin-resistant strains), Fusobacterium spp.

'Mefoxin' is active against some strains of the following bacteria: *Acinetobacter calcoaceticus* var. *anitratus (Herellea vaginicola), Acinetobacter calcoaceticus* var. *lwoffi (Mima polymorpha), Alcaligenes faecalis*, Citrobacter spp., Flavobacterium spp., Enterobacter spp.

'Mefoxin' is not active against Pseudomonas, most strains of enterococci and many strains of *Enterobacter cloacae*.

Dosage and administration

'Mefoxin' may be administered intravenously or intramuscularly (see reconstitution directions for each route below). Dosage and route of administration should be determined by severity of infection, susceptibility of the causative organism and condition of the patient.

Therapy may be started while awaiting the results of susceptibility testing.

Dosage: The usual adult dosage is 1 g of 'Mefoxin' every eight hours. (See chart.)

In adults with renal insufficiency an initial loading dose of 1 g or 2 g may be given. After a loading dose the following recommendations for maintenance dosage may be used as a guide.

Usual adult dosage

Type of infection	Dose	Frequency	Total daily dosage
Uncomplicated	1 g	Every 8 hours (occasionally every 6 hours)	3 g (4 g)
Moderately severe or severe	2 g	Every 8 hours (occasionally every 6 hours)	6 g (8 g)
Infections generally needing antibiotics in higher dosage	3 g (2 g)	Every 6 hours (Every 4 hours)	12 g

Maintenance dosage of 'Mefoxin' in adults with reduced renal function

Renal function	Creatinine clearance (ml/min)	Dose	Frequency
Mild impairment	50-30	1-2 g	Every 8-12 hours
Moderate impairment	29-10	1-2 g	Every 12-24 hour
Severe impairment	9-5	0.5-1 g	Every 12-24 hou
Essentially no function	<5	0.5-1 g	Every 24-48 hou

In the patients undergoing haemodialysis, the loading dose of 1 to 2 g should be given after each haemodialysis, and the maintenanc dose should be given as indicated in the table above.

Intravenous administration: reconstitute 'Mefoxin' with Water for Injection BP. 1 g is soluble in 2 ml. Although 'Mefoxin' is very soluble, for intravenous use it is preferable to add 10 ml of Water f Injection BP to the 1 g vial or the 2 g vial. Shake to dissolve and th withdraw entire contents of vial into syringe.

For direct intravenous injection, 'Mefoxin' may be slowly injected

Outstanding clinical benefits

Wide-ranging activity against Gram-negative and Gram-positive pathogens, anaerobes as well as aerobes.

Unusually high resistance to β-lactamase degradation.

Highly active against *Bacteroides fragilis* and strains of *E. coli*, Serratia, Klebsiella spp. and Proteus spp. (including indole positive strains) resistant to other antibacterial agents.

Offers high levels of active antibiotic in the body.

A record of relative safety comparable to the penicillins.

Prime indications include:

- Peritonitis, and other intra-abdominal and intrapelvic infections.
- Female genital tract infections
- Septicaemia
- Skin and soft tissue infections
- Bone and joint infections
- Urinary tract infections
- Respiratory tract infections (e.g. inhalation pneumonia)

Specific clinical profile

'Mefoxin' is exceptionally valuable wherever Gram-negative pathogens and anaerobes are involved in mixed infections. When mixed infections are suspected, therapy may be started while awaiting the results of susceptibility testing.

Mefoxin®

Cefoxitin sodium, MSD **INJECTION**

First of the cephamycins

the vein over a period of 3 to 5 minutes or may be given through the tubing when the patient is receiving parenteral solutions.

An intermittent intravenous infusion of 'Mefoxin' may be employed when large amounts of fluid are to be given. However during infusion of the solution containing 'Mefoxin' it may be advisable temporarily to discontinue administration of any other infusion solution at the same site (by using an appropriate IV infusion set).

A solution of 'Mefoxin' may also be given by continuous intravenous infusion (see below for compatibility and stability).

Intramuscular administration ONLY

Reconstitute 'Mefoxin' 1 g with 2 ml of Water for Injection BP or 0.5% or 1% lignocaine hydrochloride (without adrenaline) solution. 'Mefoxin' is given by deep injection into a large muscle mass. Avoid injection into a blood vessel.

Compatibility and stability

A solution of 'Mefoxin' in Water for Injection BP may be added to the following solutions: 0.9% Sodium Chloride Injection BP; 5% or 10% Dextrose Injection BP; Dextrose and Sodium Chloride Injection (5%/0.9%, 5%/0.45% or 4.3%/0.18%); Lactated Ringer's Injection USP; 5% Dextrose in Lactated Ringer's Injection; 5% or 10% invert sugar in water; 10% invert sugar in saline solution; 4.2% Sodium Bicarbonate Injection BP; M/6 Sodium Lactate Injection BP.

'Mefoxin' as constituted to 1 g/10 ml with Water for Injection BP, Water for Injection BP preserved with parabens or benzyl alcohol, 0.9% Sodium Chloride Injection BP or 5% Dextrose Injection BP maintains satisfactory potency for 24 hours at room temperature, for 96 hours under refrigeration, and for 13 weeks in the frozen state.

'Mefoxin' can be administered concomitantly with such antibiotics as gentamicin, tobramycin, carbenicillin or amikacin. When 'Mefoxin' is administered with another antibiotic, they should not be mixed in the same syringe.

Contra-indications

'Mefoxin' is contra-indicated in persons who have shown hypersensitivity to cefoxitin. In the absence of clinical experience 'Mefoxin' should not be administered to patients who have shown hypersensitivity to cephalosporins.

Precautions

There is some clinical and laboratory evidence of partial cross-allergenicity between cephamycins and other β-lactam antibiotics: penicillins and cephalosporins. Severe reactions (including anaphylaxis) have been reported with most β-lactam antibiotics.

Before therapy with 'Mefoxin', careful inquiry should be made concerning previous hypersensitivity reactions to β-lactam antibiotics. 'Mefoxin' should be given with caution to penicillin-allergic patients.

Any patient who has demonstrated some form of allergy, particularly to drugs, should receive antibiotics cautiously. If an allergic reaction to 'Mefoxin' occurs, the drug should be discontinued.

The total daily dosage should be reduced when 'Mefoxin' is administered to patients with transient or persistent reduction of urinary output due to renal insufficiency (see 'Dosage and administration') because high and prolonged serum antibiotic concentrations can occur from usual doses.

Interference with laboratory tests

A false positive reaction to glucose in the urine may occur with reducing substances but not with the use of specific glucose oxidase methods.

Use in pregnancy

Use of the drug in women of child-bearing potential requires that the anticipated benefits be weighed against the possible hazards. Reproductive and teratogenic studies have been performed in mice and rats and have revealed no evidence of impaired fertility or harm to the foetus due to 'Mefoxin'. There are no well-controlled studies with 'Mefoxin' in pregnant women.

Nursing mothers

'Mefoxin' is excreted in human milk.

Infants and children

Pending the outcome of clinical trials in progress, the conditions for use of 'Mefoxin' in infants and children remain to be defined.

Side effects

'Mefoxin' is generally well tolerated. Side effects rarely require stopping treatment and usually have been mild and transient.

Local reactions

Thrombophlebitis has occurred with intravenous administration. Pain, induration and tenderness after intramuscular injections have been reported.

Allergic

Maculopapular rash, urticaria, pruritus, eosinophilia, fever and other allergic reactions have been noted rarely.

Gastro-intestinal

Diarrhoea has been reported rarely.

Blood

Transient leucopenia and neutropenia have been reported. Some individuals, particularly those with azotaemia, may develop positive direct Coombs tests during therapy with 'Mefoxin'.

Liver function

Transient elevations in SGOT, SGPT, serum LDH and serum alkaline phosphatase have been reported rarely.

Kidney

Elevations in serum creatinine and/or blood urea levels have been observed. The role of 'Mefoxin' in changes in renal function tests is difficult to assess, since factors predisposing to pre-renal azotaemia or to impaired renal function usually have been present.

Presentation

In vials containing 1 g or 2 g of cefoxitin as the sodium salt. Each gram of cefoxitin contains approximately 51.5 mg (2.2 mEq) sodium.

Product licence numbers: 1 g 0025/0130, 2 g 0025/0131.

Product authorisation numbers: 1 g PA 35/42/1, 2 g PA 35/42/2.

Issued October 1978.

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Basic NHS cost is £4.21 for the 1 g vial and £8.40 for the 2 g [illegible]

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Androgen secretion by human adrenal and testis cells in culture.
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Round Table: Adrenal androgens—the future.

Speakers:

G. Abraham (Rolling Hills, USA)
D. Anderson (Manchester, England)
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G. Giusti (Florence, Italy)
P. MacDonald (Dallas, USA)
A Neville (Sutton, England)
B. Nordin (Leeds, England)
W. Odell (Torrance, USA)
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Official language: English

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1. J. int. med. Res. (1977) 5 (Suppl. 3): 86-91.
2. ibid., 101-108. **3.** ibid., 98-100.
4. ibid., 114-123.

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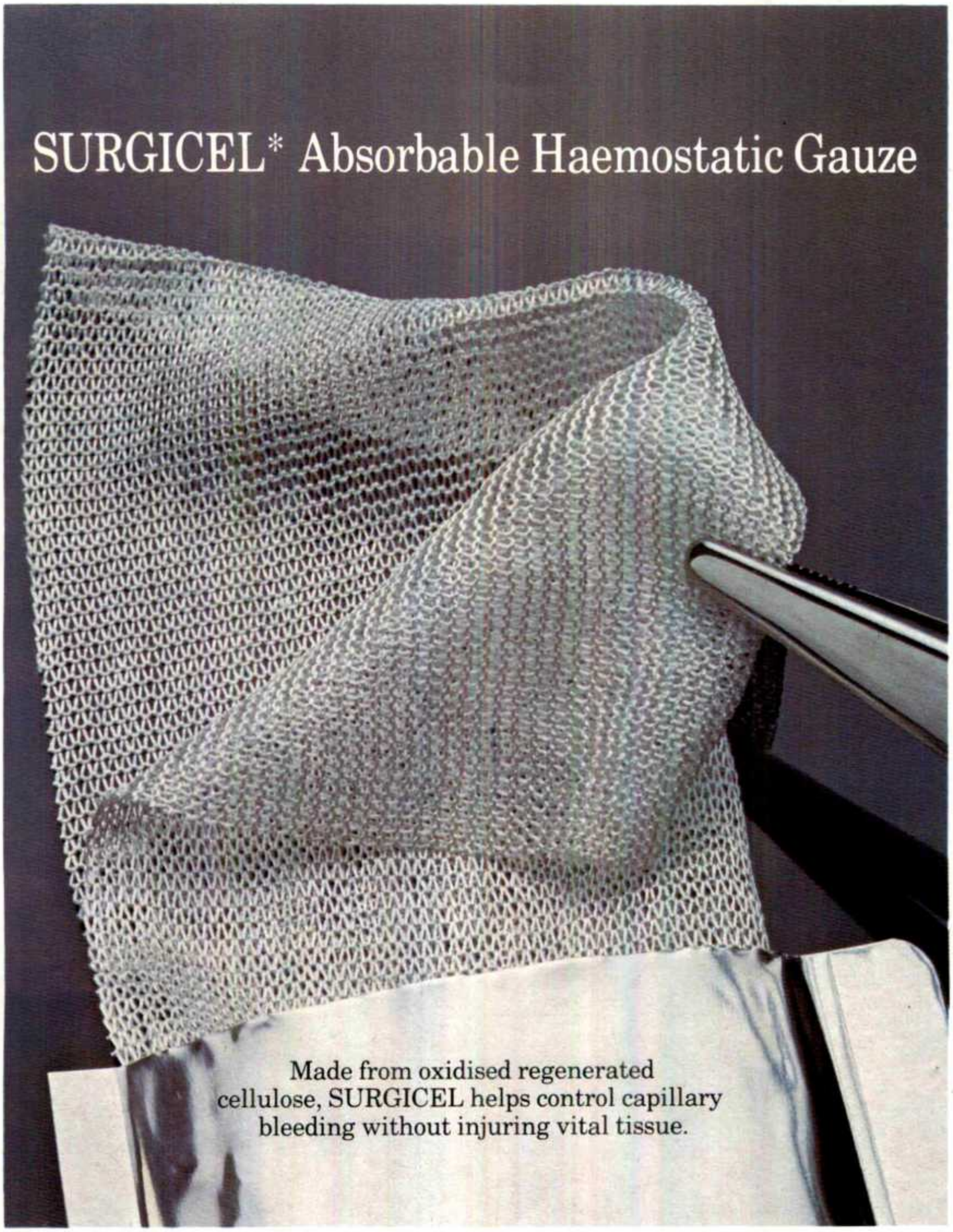
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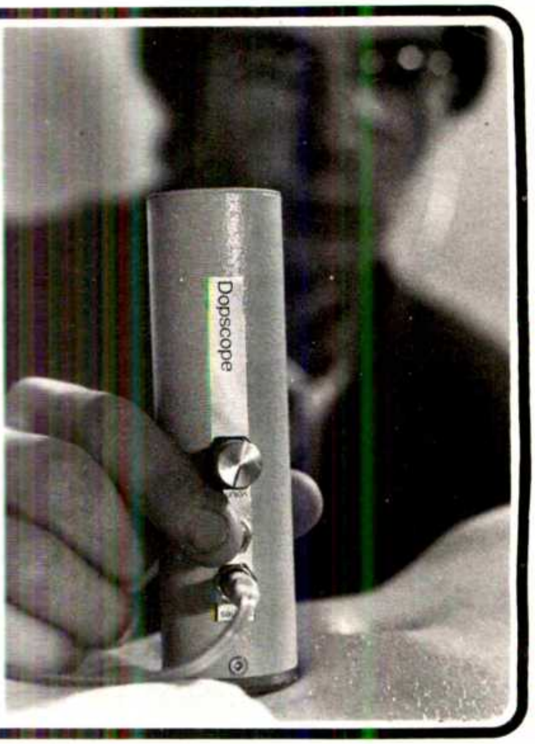

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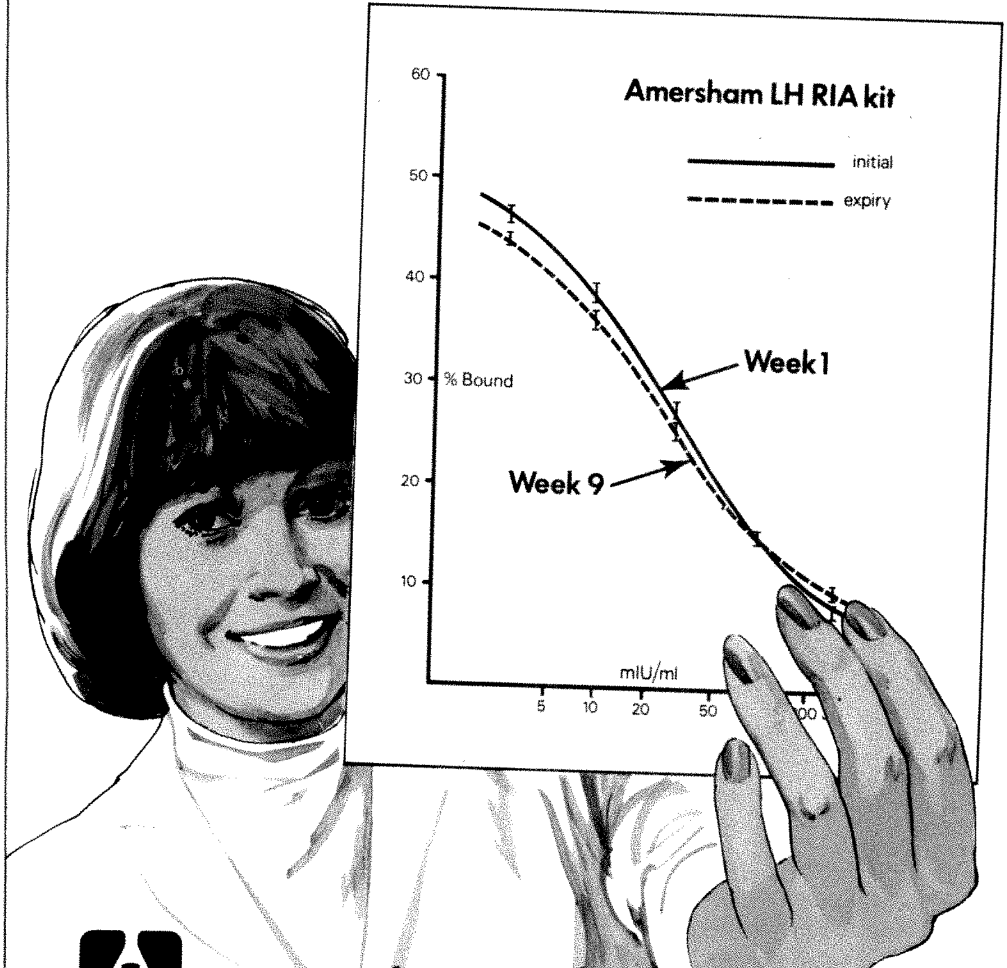

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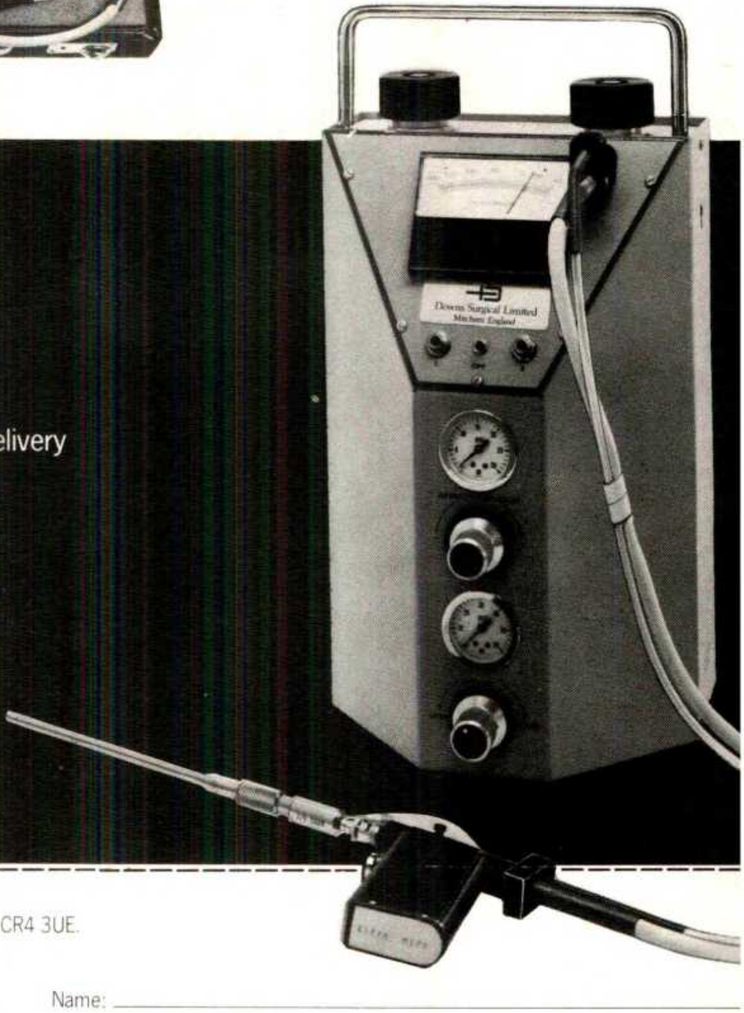

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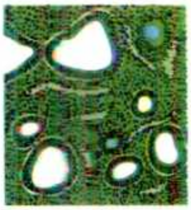

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1. *Modern Medicine* 45, 10, 1977. **Presentation:** Each memo-pack holds 11 white tablets each containing 2mg oestradiol valerate, and 10 orange tablets each containing 2mg oestradiol valerate and 0.5mg norgestrel. **Uses** The treatment of the climacteric syndrome. Cyclo-Progynova does not consistently inhibit ovulation and should not be relied upon for contraception. **Dosage:** *First course.* Patients still menstruating should begin on th 5th day of menstruation. Others may begin at any time. One white tablet is taken daily for 11 days, followed by one orange tablet for 10 days. *Subsequent courses.* An interval of 7 days, during which bleeding is to be expected precedes each subsequent course. **Contra-indications:** Pregnancy; severe disturbances of liver function; history during pregnancy of jaundice, herpes, general pruritus or deterioration of otosclerosis; past or presen thrombotic disorders; sickle-cell anaemia; existing or treated oestrogen-dependent tumours; endometriosis; mastopathy; disordered lipid metabolism; Dubin-Johnson and Rotor syndromes. **Warnings:** Hormona contraception should be stopped when Cyclo-Progynova is started, and the patient be advised to take non-hormonal contraceptive precautions. Any irregular bleeding should be investigated. **Possible side-effects** Anxiety, increased appetite, bloating, breast and cardiac symptoms, depression, dizziness, leg pains and swelling, altered libido, nausea, rashes, vomiting and altered weight. Exacerbation of diseases known to worse during pregnancy. **Reasons for immediately stopping treatment:** If migrainous or frequent and unusually severe headaches occur for the first time; if there are other symptoms that are possible prodromat of vascular occlusion; if trauma, illness or impending surgery is considered to entail a risk of thrombosis; if jaundice, pregnancy or significant hypertension occurs. **Special information:** Examination of th pelvic organs, endometrium, breasts and blood pressure is advised before and periodically during treatment with Cyclo-Progynova. **Basic NHS price** £2.24 per memo-pack. **Product licence number** 0053/005

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British Journal of Obstetrics and Gynaecology

Vol 86 No. 3 NEW SERIES MARCH 1979

CLINICAL DILEMMAS ARISING FROM THE ANTENATAL DIAGNOSIS OF NEURAL TUBE DEFECTS

BY

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Summary

Eight case histories are presented which demonstrate clinical problems associated with the antenatal screening for and diagnosis of neural tube defects. It is suggested how some of these problems might be avoided in future.

The clinical and personal problems of pregnant patients screened for congenital abnormalities are necessarily submerged in reviews (e.g. Ferguson-Smith *et al*, 1978; Brock *et al*, 1978; and Wald *et al*, 1979). As the critical period for diagnosis is short, considerable anxiety and tension can be experienced by both doctor and patient.

This paper presents chronologically eight case histories of selected patients. They demonstrate clinical dilemmas and pitfalls which can arise in the antenatal screening for and diagnosis of neural tube defects (NTDs). Four of the patients were from the 6443 who were screened for NTDs in Oxford from May 1975 to the end of 1977, and are here given the same reference numbers as in our review of screening over this period (Wald *et al*, 1979). Two patients (No. 7 and 8) were screened after the end of 1977, and two others (No. 2 and 3) had an amniocentesis for reasons other than a high maternal serum alpha-fetoprotein (AFP).

CASE HISTORIES AND DISCUSSION

Patient No. 1 (C1)

History. The patient, para 0+0, was aged 29 years. The serum AFP at 15 weeks from the first day of the last menstrual period was 10·8 multiples of the median. An ultrasound scan at 16 weeks showed a gestation which was consistent with menstrual dates. An amniocentesis at 16 weeks showed an AFP level of 602 µg/ml (93·7 SDs above the mean). The amniotic fluid was slightly blood-stained with less than 5 per cent fetal red cells (using the Kleihauer technique); the total red cell count was not done. A diagnosis of neural tube defect was made and at 17 weeks a termination of pregnancy was done. The amniotic fluid AFP level at termination was above 600 µg/ml or more than 100 SD above the mean. No abnormality was found in the fetus after an autopsy examination which included electron microscopy studies of the kidneys.

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Discussion. The distress produced in this patient was compounded by the fact that the antenatal diagnosis of neural tube defect had been made with confidence. Fetal abnormalities other than NTDs known to be associated with raised amniotic fluid AFP levels, such as congenital nephrosis, oesophageal atresia, and Turner's syndrome, were considered and sought. The electron microscopy findings were not regarded as indicative of congenital nephrosis, but amniotic fluid albumin measurements were consistent with this diagnosis (6·0 and 8·0 mg/ml respectively) in the two amniotic fluid samples collected. The amniotic fluid albumin level in a case of congenital nephrosis previously reported (Thom *et al*, 1977) was similar, namely 6·8 mg/ml (H. Thom, personal communication). In our own patient, no fetal urine could be obtained and it was not therefore possible to determine whether proteinuria was present. Rapidly-adherent amniotic fluid cells, thought to be indicative of fetal NTDs (Sutherland *et al*, 1975) were not noted although routine quantitative measurements (Bobrow *et al*, 1978) were only introduced after this patient was treated.

Patient No. 2

History. The patient, para 3+0, was aged 39 years. An ultrasound scan at 16 weeks showed a gestational age which was consistent with dates and a possible posterior fibroid. An amniocentesis was done because the patient had previously given birth to a baby with Down's syndrome. Clear amniotic fluid obtained by amniocentesis at 16 weeks showed an AFP level of 50 µg/ml (4·7 SD above the mean using current normal ranges, but between 5 and 6 SD using normal ranges available during this pregnancy). There were no rapidly-adherent cells. Because the diagnosis was uncertain a repeat amniocentesis was done at 17 weeks and clear amniotic fluid obtained with an AFP level of 65 µg/ml (8·1 SD above the mean) but, again, no rapidly-adherent cells. It was therefore

TABLE I

Details relating to Patient No. 3

	Maternal blood		Amniotic fluid				
			AFP concentration				
Gestation (completed weeks)	Serum AFP (multiple of the median)	No. of fetal cells/50 low power fields	µg/ml	(SD above the mean)	RBC count (cells/mm³)	$\frac{\text{Fetal RBC}}{\text{maternal RBC}}$ (per cent)	No. of rapidly adherent cells/10 high power fields
16	1·4	—	—	—	—	—	—
16	pre-amniocentesis 1·2 post-amniocentesis 16·4	nil nil	70	7·9	31,500	'mainly fetal'	7
17	pre-amniocentesis 5·9	20	64	7·9	18,000	85	103
18	pre-amniocentesis 4·5	15	35	3·6	13,800	93	124
20	pre-amniocentesis 2·9	nil	21	2·2	—	—	58

thought possible that the fetus had an NTD and the patient chose to have a termination of pregnancy. One normal fetus and one fetus papyraceous were expelled from the uterus.

Discussion. In this patient the border-line high amniotic fluid AFP levels may have been due to the presence of a dead twin which was ultimately delivered as a fetus papyraceous at termination of pregnancy.

Patient No. 3

History. The patient, para 0+0, was aged 24 years. An ultrasound scan at 16 weeks showed a maturity consistent with menstrual dates and a left ovarian cyst which was removed at laparotomy at 20 weeks. An amniocentesis was done (before the laparotomy) because the patient's husband had spina bifida occulta. The first amniotic fluid sample was heavily blood-stained and three further amniocenteses were done. Table I shows their timing and results. The patient accepted the advice to proceed with her pregnancy and ultimately was delivered of a normal infant.

Discussion. This patient illustrates the way in which blood-staining of amniotic fluid complicates diagnosis. The high level of amniotic fluid AFP at the first amniocentesis was presumably due to the contamination of the fluid by fetal blood. Assuming a fetal haematocrit of 40 per cent, a fetal red cell count of 2·5 million/mm^3 and a fetal serum AFP concentration of 2·5 mg/ml, the fetal blood contamination of the first amniotic fluid sample was equivalent to an AFP elevation of 2 to 3 SD above the mean, indicating that the AFP level in the uncontaminated amniotic fluid might have been normal. One week later the level was still high. It was not until the third amniocentesis, two weeks after the first, that the true level of the amniotic fluid AFP became apparent, and this was confirmed by the fourth amniocentesis. It would appear that after a major fetal bleed into the amniotic fluid a few weeks are needed before the AFP level returns to normal. It is noteworthy that no decrease in the AFP level was demonstrated between the first and second samples, and this may have been due to continued fetal bleeding after the first amniocentesis.

Patient No. 4 (C2)

History. The patient, para 1+1, was aged 38 years and the current pregnancy was unplanned. The menstrual dates were very uncertain and an ultrasound scan suggested a gestational age of 14 weeks. The serum AFP level at 17 weeks by ultrasound was 5·7 multiples of the median, and because of this an amniocentesis was done at this time. The clear amniotic fluid had an AFP level of 59 μg/ml (7·1 SD above the mean) and there were no rapidly-adherent cells. Because the diagnosis was uncertain a repeat amniocentesis was done at 18 weeks by ultrasound. The amniotic fluid AFP was 85 μg/ml (12 SD above the mean). The fluid was slightly blood-stained, the red cell count less than 5/mm^3, 12 per cent of cells being fetal. No rapidly-adherent cells were present, and because of this the patient was advised to continue with the pregnancy. She requested termination and the fetus was normal.

Discussion. The medical advice to the patient was that it would be best to continue with the pregnancy. She had earlier discussed termination of pregnancy on social grounds with her family doctor but had decided against this. However, the new uncertainty as to whether the baby was normal made her reconsider her original decision.

Patient No. 5 (D1)

History. The patient, para 0+0, was aged 26 years. The serum AFP at 18 weeks was 1·3 multiples of the median and was therefore not reported as abnormal. An ultrasound scan was done at 24 weeks by dates in the course of routine antenatal care, and suggested a gestational age of 19 weeks. By revised gestational age the serum AFP had therefore been high at 2·5 multiples of the median for 13 weeks. Pregnancy continued and ultimately the patient was delivered of a child with an open spina bifida who died shortly after birth.

Discussion. This patient showed how important is accurate dating of gestation. She had an ultrasound scan because, by the size of her uterus, it was thought that she might be less advanced in pregnancy than her dates suggested. The ultrasound scan was therefore performed late in the pregnancy for reasons unconnected with AFP screening and the result was not linked to the AFP result. If it had been done earlier and the two investigations linked this patient would probably have been asked to have a second AFP test at a more appropriate time. Since the AFP level at 13 weeks, using the estimated gestational age based on the ultrasound scan, was fairly high it is likely that a later screening test would have been positive.

Patient No. 6 (B8)

History. The patient, para 0+0, was aged 22 years. The serum AFP at 16 weeks by certain dates was 2·6 multiples of the median. An ultrasound scan at 21 weeks suggested a gestational age of 18 weeks, which would have made the serum AFP 3·8 multiples of the median at the time of testing. An amniocentesis was therefore done at 21 weeks by dates. The amniotic fluid AFP level was 45 µg/ml (7·8 SD above the mean by dates, 5·5 SD above the mean by ultrasound scan). The fluid was blood-stained, the red cell count 12 000/mm^3, 15 per cent of cells being fetal. There were no rapidly-adherent cells. The diagnosis was confused by the blood-staining of the amniotic fluid and a repeat amniocentesis was done at 22 weeks by dates. The amniotic fluid AFP level was 48 µg/ml (9·4 SD above the mean by dates, 6·8 SD above the mean by ultrasound scan). The fluid remained blood-stained, the red cell count 20 000/mm^3, 10 per cent of cells being fetal. There were 6 rapidly-adherent cells/10 high power fields, an equivocal result. An NTD was thought possible but pregnancy continued. The baby had an open spina bifida which was closed surgically and a Spitz-Holter valve was inserted to control hydrocephaly. The infant has some lower limb weakness.

Discussion. The amniotic fluid AFP level from the first amniocentesis was not considered high enough to justify a firm diagnosis if the ultrasound dating of gestation was used, and this together with the fact that the fluid was blood-stained, prompted the decision to perform a second amniocentesis. Although the second sample of amniotic fluid had a higher AFP level, the amount of fetal blood as well as the absence of rapidly-adherent cells in the first amniotic fluid sample led to a decision not to recommend termination, a procedure that the couple themselves were reluctant to consider. According to the date of the spontaneous delivery and the paediatric assessment of the newborn infant, it seemed likely that the gestational age had been correctly estimated from the last menstrual period. There was therefore little doubt that the amniotic fluid AFP results were abnormal. Thus, one should take heed when a patient is certain of the date of her last menstrual period. In retrospect, the diagnosis of NTD should have been firmer.

Patient No. 7

History. The patient, para 0+0, was aged 28 years and had very uncertain dates. An ultrasound scan suggested a gestational age of 19 weeks and the serum AFP level at this time was 3·0 multiples of the median for 19 weeks. Pregnancy continued and the infant was born with an exomphalos which was operated on successfully; no residual disability is expected.

Discussion. By mistake, the expected date of delivery was entered on the AFP request card instead of the date of the last menstrual period. When the AFP result was interpreted in the laboratory it was clear that the date was wrong. It was assumed that the last menstrual period has been given but that the wrong year had been entered. The mistake was 'corrected' by making the date one year earlier, so that the test was taken as having been performed at 23 weeks of gestation and the result was considered normal at 1·6 multiples of the median. An ultrasound scan examination performed on the same day as the AFP test indicated that the gestational age of the patient was 19 weeks. However, since only patients with high serum AFP tests had the result re-interpreted in the light of an ultrasound scan, the mistake did not come to light until after delivery. If the correct date had been entered on the request card the patient would have had a serum AFP value above the cut-off level. This patient not only identifies the consequences of clerical error but also provides an example of one of the ethical dilemmas associated with AFP screening. Some cases of fetal exomphalos are associated with raised serum and amniotic fluid AFP levels, and are therefore likely to result in a termination even though the defect can sometimes be surgically repaired. However, the frequency of this malformation in the absence of NTD is only about 0·1 per 1000 births (Butler and Alberman, 1969) and it is unlikely that all cases will be detected by screening. Therefore, the chance of detecting a pregnancy in which a potentially viable infant has an exomphalos is likely to be very small.

Patient No. 8

History. The patient, para 1+0, was aged 29 years. At 16 weeks the serum AFP level was 4·2 multiples of the median. An ultrasound scan at 19 weeks by dates revealed a poorly outlined fetal head consistent with a gestational age of only 15 weeks. Amniocentesis was recommended because of a high serum AFP but no amniotic fluid was obtained. A repeat amniocentesis at 20 weeks by dates produced very viscous amniotic fluid with an AFP level of more than 7000 μg/ml (more than 1000 SD above the mean), and there were no rapidly-adherent cells. It was decided to recommend a termination of pregnancy, and this was done at 21 weeks by dates; the fetus had an open spina bifida, encephalocoele and an exomphalos.

Discussion. The second amniocentesis yielded very viscous fluid with a very high amniotic fluid AFP level. It is likely that the fluid came from the encephalocoele sac. Amniotic fluid collected at the autopsy had an AFP level of 89 μg/ml (17·5 SD above the mean), well above normal but much less than the other level obtained. At the same time the concentration of AFP in the fluid aspirated from the encephalocoele was about 2000 μg/ml (436 SD above the mean), of the same order of magnitude as was found in the fluid collected at amniocentesis.

CONCLUSION

One patient (No. 8) is an example of a surprising outcome rather than a particular clinical problem. The exceedingly high AFP level in what was initially thought to be amniotic fluid but later judged to be encephalocoele fluid did not lead to an incorrect course of management. The patient is described to show how a high AFP level may be due to fluid being other than of amniotic origin.

The remaining seven patients highlight problems associated with antenatal screening and diagnosis for neural tube defects, some avoidable and some not so. Problems associated with patients No. 1, 2 and 4 were probably unavoidable. No. 1 was an example of a false-positive with very high serum and amniotic fluid AFP levels and abnormal amniotic fluid albumin levels consistent with the presence of a fetal renal lesion. Such cases are likely to occur again in the future; while the frequency of false-positives is still not precisely known it is probably less than 0·1 per cent at such high AFP levels. There is also perhaps no effective remedy for the kind of unnecessary termination carried out in patient No. 2 though ultrasound scanning may identify a dead fetus alongside a normal twin (Letchworth *et al*, 1977) and, in the future, technical improvement in ultrasound may make the detection of such pregnancies easier. Patient No. 4 is another example of an amniotic fluid AFP false-positive against which there is at present no adequate safeguard. The absence of rapidly-adherent cells influenced the decision not to advise termination, but we know that their absence cannot exclude open spina bifida (e.g. the first amniotic fluid sample from patient No. 6) and, in addition, a normal outcome can be associated with large numbers of these cells (e.g. patient No. 3).

Patients No. 3, 5, 6 and 7 could probably be managed more satisfactorily in the light of more recent information. Patient No. 3 is an example of the complications associated with the interpretation of an AFP level in blood-stained amniotic fluid. It would seem that about two weeks should elapse before a second amniocentesis is performed. Patient No. 5 shows the importance of the accurate estimation of gestational age. Patient No. 6 shows how recent clinical experience and early research findings (in this example those concerning rapidly-adherent cells) influenced too much the clinical management of a patient in whom the diagnosis of NTD should have been fairly clear. After this it was agreed that the detection of rapidly-adherent cells would be used for research and not for clinical purposes. Patient No. 7 shows how easily clerical errors can be made and how serious they may be. In Oxford only positive results are reported: if this was widened to include negative results the chance of clerical errors would be considerably increased.

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A COMPARISON OF PGE_2 AND $PGF_{2\alpha}$ VAGINAL GEL FOR RIPENING THE CERVIX BEFORE INDUCTION OF LABOUR

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Summary

The efficacy of a vaginal gel containing either 5 mg prostaglandin E_2 (PGE_2) or 25 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to ripen the unfavourable cervix for labour induction was assessed in a double blind trial. $PGF_{2\alpha}$, compared with PGE_2, had little effect upon the clinical state of the cervix, but the resultant duration of labour in each of the two groups was shorter than in a control group. While the numbers of patients requiring oxytocin stimulation of labour and regional analgesia were reduced in both groups compared with the controls, $PGF_{2\alpha}$ was much less effective than PGE_2. Uterine hypertonus was observed using both prostaglandins during an experimental study and the implications are discussed.

Virtually all the published work on the use of prostaglandins for cervical ripening prior to induction of labour in the human has involved the administration of prostaglandin E_2 (Calder *et al*, 1977; Thiery *et al*, 1977; Shepherd *et al*, 1976; MacKenzie and Embrey, 1977). By contrast, in most of the similar animal studies prostaglandin $F_{2\alpha}$ has been used (Fitzpatrick, 1977; Liggins *et al*, 1977).

Although almost all the published results indicate that prostaglandin E_2 (PGE_2) is efficient for the purpose of ripening the cervix, it seemed prudent to examine the comparative value of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in this respect.

Patients and Methods

All patients studied had medical or obstetric indications for labour induction and gave informed consent. Each had unfavourable induction prospects as designated by a modified Bishop score of 0 to 3. All were primigravidae with single fetuses in cephalic presentations at gestations ranging from 37 to 43 weeks.

Preparation of the prostaglandin gel has been described (MacKenzie and Embrey, 1977). In each instance, 10 ml of a 5 per cent solution of methyl hydroxy ethyl cellulose (Tylose MH 300: Hoechst) gel was used containing either 5 mg PGE_2 (Prostin E_2: Upjohn) or 25 mg $PGF_{2\alpha}$ (Dinaprost $F_{2\alpha}$: Upjohn).

Before the comparative trial, the effect of PGE_2 and $PGF_{2\alpha}$ on uterine activity was studied by intrauterine pressure monitoring using a transcervical extra-amniotic catheter system connected to a strain gauge pressure transducer and a hot wire pen recorder (Hewlett-Packard 2703A); fetal heart rate was continuously recorded using an external ultrasound head. A number of patients in the trial were monitored by external tocographic methods.

Subsequently a total of 48 patients were randomly allocated to treatment with gel containing either PGE_2, or $PGF_{2\alpha}$ or gel alone and the study was double blind. Cervical assessment and vaginal treatment were done by the same person between 1600 and 1900 hours in the evening before planned induction of labour. Fetal and maternal observations were made over the subsequent four to five hours. On the following morning, those patients who were not in labour had an amniotomy and started an intravenous infusion of oxytocin at 0830 to 0930 hours. Subsequent labour was managed by the duty obstetric staff.

Results

Table I summarizes results in the two treatment groups and the control group. In each of the three groups, the cervical score had significantly improved when assessed the following morning ($p < 0{\cdot}01$; Wilcoxon signed ranks test). The mean length of labour was significantly shorter in the two groups treated with prostaglandins compared with the controls ($p < 0{\cdot}025$; Student's 't' test), while those treated with PGE_2 and given an epidural required less anaesthetic than the controls ($p < 0{\cdot}05$). There were no other significant differences between the three groups for the other indices calculated.

Delivery by Caesarean section for failure to progress in labour and achieve adequate cervical dilatation was necessary for one patient in each of the treatment groups. Three patients were delivered by Caesarean section in the control group, 2 patients for fetal distress and one for failure to progress in labour.

From the initial observations made, tocographic recordings of patients receiving $PGF_{2\alpha}$ were essentially similar to those that we have previously reported using PGE_2 (MacKenzie and Embrey, 1977). Usually within two hours of prostaglandin instillation, uterine contractions commenced and occurred at 1 to 3 minute intervals with a maximum intrauterine pressure of 30 to 40 mm Hg; contractions either waned after 4 to 5 hours or progressed to established labour. On one occasion during the initial study period using 5 mg of PGE_2 and on one occasion using 25 mg of $PGF_{2\alpha}$ with a transcervical intrauterine monitoring catheter *in situ*, uterine hypertonus occurred. In each instance this was readily evident clinically and both patients were delivered by emergency Caesarean section; both the infants were in good condition at delivery, though one had shown previous fetal heart rate changes indicative of acute fetal distress.

TABLE I

Results of patients treated with PGE_2 gel or $PGF_{2\alpha}$ gel or placebo gel before labour induction (figures quoted are total patients or Means ± SD)

Treatment group	5 mg PGE_2 gel (n = 15)	25 mg $PGF_{2\alpha}$ gel (n = 16)	Gel alone (n = 16)
Gestation (weeks)	40·1 ± 1·3	39·8 ± 1·3	40·4 ± 1·3
Cervical score: Before treatment	2·50 ± 0·6	2·50 ± 0·5	2·56 ± 0·6
After treatment*	10·87 ± 3·3	4·69 ± 3·6	4·06 ± 1·4
No. with a score of under 5 after treatment	0	11	12
No. in established labour after treatment	11	2	0
Length of labour (hours)	8·6 ± 3·3	10·6 ± 4·3	14·8 ± 5·3
No. in labour more than 12 hours	3	7	10
No. in labour more than 18 hours	0	1	6
No. requiring intravenous oxytocin	6	14	16
No. given epidural analgesia	7	11	13
Total dose of anaesthetic† (ml)	18·0 ± 13·2	22·7 ± 10·7	34·8 ± 19·8
Apgar score of less than 5 at one minute	1	1	2
Apgar score at one minute	7·6 ± 2·0	7·9 ± 1·7	7·6 ± 2·2
Apgar score at five minutes	9·6 ± 1·2	9·2 ± 1·8	9·1 ± 2·0
Infant birth weight (g)	3240 ± 427	3348 ± 334	3369 ± 463

* Patients established in labour have a cervical score of 13.

† 0·5 per cent Bupivacaine hydrochloride.

Discussion

Compared with the control group, there was little evidence that $PGF_{2\alpha}$ influenced the clinical state of the cervix. In these two groups, 11 and 12 patients had cervical scores of less than 5 at labour induction. All the patients treated with PGE_2, however, had shown considerable improvement in cervical ripeness. A possible explanation for the difference between the two prostaglandins is one of comparative dosage. In numerous previous studies concerning the uterotonic effects of prostaglandins, PGE_2 has been found to be 5 to 10 times more potent than $PGF_{2\alpha}$ (Embrey, 1975). However, while it is conceivable that the dose of $PGF_{2\alpha}$ was too small compared with that for PGE_2, the results observed for the former were still far inferior to those previously reported using a double blind comparison of 2 mg PGE_2 gel and gel alone (MacKenzie and Embrey, 1977). Also, it is significant that in the present study there was no obvious clinical or tocographic difference to be discerned in the uterine activity provoked in the two groups of patients.

While the post-treatment cervical score was little influenced by $PGF_{2\alpha}$, the subsequent mean length of labour in this group was significantly shorter than in the control group ($p < 0{\cdot}025$); the reduction in the mean length of labour was even greater in the PGE_2 treated group. Such observation suggests that the two prostaglandins under investigation may have different actions upon the pregnant uterus at term; possibly PGE_2 has a more specific action upon the cervix promoting effacement and dilatation, while the action of $PGE_{2\alpha}$ is primarily upon the myometrium. Animal studies, however, do not fully support this hypothesis. Liggins *et al* (1976) found that intra-aortic infusions of $PGF_{2\alpha}$ in sheep ripened the long tubular cervix in 24 to 48 hours even when there was no uterine activity; but repetition of this study by Fitzpatrick (1977) failed to produce similar results. Hutton and Liggins (unpublished observations) have also noted ripening effects on the human cervix following an intravenous infusion of $PGF_{2\alpha}$; to date, however, there is very little published work on the clinical effect of $PGF_{2\alpha}$ for this purpose, though Lindberg (1977) reported poor results when inducing labour with intravenous $PGF_{2\alpha}$ when the cervix was unripe.

Attempts to demonstrate histological changes in the cervix following prostaglandin treatment have been unrewarding apart from detecting the presence of oesinophilia (Liggins, 1977). Oestradiol can modify cervical collagen in certain animals (Leppi and Kinnison, 1971) and clinical studies support a relationship between circulating oestradiol levels and prostaglandin efficacy in the cervical ripening effect (MacKenzie *et al*, 1979). Whether PGE_2, in ripening the cervix, can induce similar microscopic changes in cervical morphology in the human has yet to be shown.

We have previously shown (MacKenzie and Embrey, 1978) that those patients who go into labour following PGE_2/gel treatment alone have a reduced need for intravenous oxytocin, epidural analgesia, and lower rates of operative delivery. These results are corroborated in the present study. Although no significant differences were apparent in the state of the newborn, Calder *et al* (1977) have shown convincingly that ripening the cervix with PGE_2 prior to labour induction reduced the incidence of asphyxia at birth. We have failed to show any similar benefit from $PGF_{2\alpha}$ but with larger numbers this might become evident.

Of major concern is the observation of hypertonus in two patients, one treated with PGE_2 and one with $PGF_{2\alpha}$. From the literature, the incidence of hypertonus after prostaglandin would appear to be no greater than that observed in spontaneous or oxytocin induced labour. Both our patients had an intrauterine catheter *in situ* for pressure recording; in over 800 patients treated in our unit with vaginal prostaglandins without an intrauterine catheter *in situ*, only one similar episode has been noted (MacKenzie and Embrey, 1978). It is possible that the intrauterine catheter carried prostaglandin gel through the cervical canal into the extra-amniotic space, in an amount which was excessive for the responsive myometrium.

Our results indicate that in the human, while some reduction in the length of labour can be achieved if $PGF_{2\alpha}$ is given before induction, the clinical state of the cervix does not change and there is little improvement in the outcome of induced labour. By contrast, PGE_2 appears to achieve these objectives.

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THE RELATION BETWEEN PLASMA OESTROGEN, PROGESTERONE AND PROLACTIN CONCENTRATIONS AND THE EFFICACY OF VAGINAL PROSTAGLANDIN E_2 GEL IN INITIATING LABOUR

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Summary

In an attempt to relate the efficacy of treatment to endogenous hormone levels, plasma oestrogen, progesterone and prolactin levels were analysed in women at term following the vaginal administration of prostaglandin E_2 gel to induce labour. Patients who went into labour after treatment had significantly higher oestradiol-17β levels before treatment compared with those requiring formal surgical induction the following day. No difference could be demonstrated in levels of progesterone or prolactin in the two groups of patients. The significance of these results is discussed.

The state of the uterine cervix can be improved and the subsequent labour prospects made more favourable by the instillation of a viscous solution of prostaglandin E_2 (PGE_2) into the posterior vaginal fornix the night before planned induction of labour (MacKenzie and Embrey, 1977 and 1978). It is unclear how PGE_2, used in this way, alters the state of the cervix or leads to the initiation of parturition. Although the onset of labour after prostaglandin treatment was related to the state of the cervix, it was not possible to predict which patients would start labour.

In an attempt to elucidate the possible mechanisms involved, and thus obtain information on how the technique could be made to yield better results, we studied and compared hormone levels in those patients who went into labour and those who did not.

PATIENTS AND METHODS

A total of 87 patients with singleton viable pregnancies and a fetal cephalic presentation was studied. The patients were to have an induction of labour for a medical and/or obstetric reason, the majority having an unfavourable cervix (Calder *et al*, 1977). Each gave her informed consent to the investigation. Sixty-nine patients whose gestation (mean ± SD) was $277{\cdot}9 \pm 8{\cdot}9$ days with varying degrees of cervical ripeness were treated with intra-vaginal PGE_2 gel between 1600 and 1900 hours on the evening before planned induction of labour: 40 primigravidae were given 5·0 mg PGE_2 and 29 multigravidae 2·5 mg PGE_2, both in a 5 per cent viscous solution of aqueous methyl-hydroxy-ethyl-cellulose gel (MacKenzie and

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Embrey, 1978). Before prostaglandin administration, a 10 ml sample of peripheral venous blood was collected into heparinized tubes and the plasma separated and deep frozen. The following morning, before amniotomy if labour had not started, a further venous blood sample was collected. Eighteen patients, (10 primigravidae and 8 multigravidae) whose gestational age (mean ± SD) was 281·6 ± 7·9 days, were used as controls: all had favourable cervical features. Venous blood samples were taken between 1600 and 1900 hours the evening before planned induction, but no PGE$_2$ was given. The following morning, before amniotomy, a further venous blood sample was taken. In all cases, labour, whether resulting from the PGE$_2$ gel alone or subsequent induction, was managed by the duty obstetric staff: the birth weight of each newborn infant was recorded.

All plasma samples were treated in the same way, and were assayed at the completion of the clinical studies. Plasma progesterone, oestradiol-17β, and prolactin were measured by specific radioimmunoassays using previously described procedures (Challis *et al*, 1973; Huang *et al*, 1971). For progesterone and oestradiol the solvent blanks were equivalent to 0·03 ± 0·03 ng/ml and 2·4 ± 3·8 pg/ml (mean ± SD) respectively. The interassay recovery of progesterone from a 0·5 ng/ml pool and of oestradiol from a 250 pg/ml pool (n = 6) was 0·46 ± 0·07 ng/ml and 244 ± 31 pg/ml (n = 14) (mean ± SD) respectively. Prolactin concentrations are quoted as ng/ml of NIH human prolactin standard (VLS-3). The inclusion of a high and a low reference plasma sample in each prolactin assay gave values of 14·95 ± 1·14 and 1·52 ± 0·43 ng (mean ± SD, n = 10) respectively. The cross reaction of human placental lactogen in the prolactin assay was less than 0·1 per cent.

RESULTS

Table I shows the values for gestation and infant birth weight and for plasma prolactin, oestradiol-17β, progesterone, and progesterone/oestradiol ratio, for the control group and for the total number of treated patients. There were no significant differences in the levels obtained between the two groups studied. When analysed separately, there was no significant differences between the multigravidae and primigravidae in the two groups studied for any of the indices measured.

The pretreatment values obtained for those patients who went into labour following the PGE$_2$ treatment alone, and those who had not gone into labour but required amniotomy the following morning are shown in Table II. Patients who went into labour had a significantly higher ($p < 0·0025$) level of circulating oestradiol-17β (26·71 ± 11·7 ng/ml) than those who did not (19·22 ± 7·0 ng/ml). There were no significant differences between the levels of circulating prolactin or progesterone nor in the progesterone/oestradiol ratio in those patients who went into labour compared with those that did not.

In the patients treated with PGE$_2$, in all of whom a cervical assessment was made prior to treatment, there did not appear to be any direct correlation between circulating endogenous steroid and prolactin levels and the clinical state of the cervix or the duration of labour, whether this followed amniotomy or PGE$_2$ treatment alone. However, each of four patients who required delivery by Caesarean section for delay in the first stage of labour after amniotomy had low oestradiol-17β levels with a mean level of 10·17 ng/ml (range 5·0 to 13·8 ng/ml) while the mean progesterone level at 152·9 ng/ml and prolactin at 212·0 ng/ml were within the normal range.

In the 26 patients who had been treated with

TABLE I

Hormone levels in treated and control patients before vaginal administration of prostaglandin E$_2$

	Treatment group (n = 69) Mean ± SD	Control group (n = 18) Mean ± SD
Gestation (days)	277·9 ± 8·9	281·6 ± 7·9
Birthweight (g)	3326 ± 477	3403 ± 329
Prolactin (ng/ml)	221·5 ± 107·2	222·3 ± 72·7
Oestradiol 17β (ng/ml)	23·9 ± 10·7	23·3 ± 10·4
Progesterone (ng/ml)	183·8 ± 84·8	165·2 ± 69·3
Progesterone/oestradiol ratio	8·3 ± 3·9	8·0 ± 4·6

Student's t test showed no significant differences between the treated and control groups.

TABLE II

Pretreatment hormone levels in 43 patients who went into labour and 26 patients who did not after vaginal prostaglandin E_2 treatment

	Did go into labour (n = 43) Mean ± SD	Did not go into labour (n = 26) Mean ± SD	Statistical significance*
Gestation (days)	279·5 ± 8·6	275·0 ± 8·8	$p < 0·05$
Birth weight (g)	3360 ± 415	3248 ± 579	NS
Prolactin (ng/ml)	213·2 ± 120·1	230·3 ± 92·8	NS
Oestradiol 17β (ng/ml)	26·7 ± 11·7	19·2 ± 7·0	$p < 0·0025$
Progesterone (ng/ml)	193·9 ± 90·0	167·4 ± 74·3	NS
Progesterone/oestradiol ratio	8·1 ± 4·0	8·7 ± 3·7	NS

* Student's t test

TABLE III

Effect of vaginal prostaglandin E_2 on hormone levels in 26 patients who did not go into labour

	Before treatment with PGE_2 Mean ± SD	After treatment with PGE_2 Mean ± SD
Prolactin (ng/ml)	230·3 ± 92·8	240·0 ± 107·1
Oestradiol 17β (ng/ml)	19·2 ± 7·0	20·73 ± 5·5
Progesterone (ng/ml)	167·4 ± 74·3	168·4 ± 74·6
Progesterone/oestradiol ratio	8·7 ± 3·7	7·9 ± 2·8

Student's t test showed no significant differences between the two groups.

PGE_2 gel, and who had not commenced labour before planned amniotomy, there were no significant differences in the levels of plasma oestradiol-17β, progesterone, and progesterone/oestradiol ratio, between the samples taken before treatment and those taken on the following morning before induction by amniotomy (Table III).

DISCUSSION

The mechanism by which prostaglandins initiate labour in the human is unclear, as is their relationship to the endocrine milieu associated with parturition. The results presented suggest that circulating oestradiol possibly acts as a mediator for the action of prostaglandin in the process of parturition rather than the reverse. It has been shown that the administration of synthetic oestradiol into the uterus can influence the state of the cervix and also the outcome of subsequent induced labour (Gordon and Calder, 1977). Of particular interest in the present study is the observation that the administration of PGE_2, in the doses used, did not influence the circulating endogenous levels of oestradiol-17β in the peripheral blood.

Gordon and Calder (1977) showed that local oestradiol instilled through the endocervical canal influenced the state of the cervix. In our study, however, there was no obvious correlation between plasma oestradiol levels and the state of the cervix either before prostaglandin treatment or the following morning in those patients who had not gone into labour. It may be that the levels measured in the peripheral circulation do not reflect the changes that had occurred locally as a result of the prostaglandin administration.

The influence that progesterone has upon the onset of parturition is continually being questioned. The concept of a progesterone block being withdrawn to allow labour to commence has been suggested (Csapo, 1956; Csapo *et al*, 1971). Comparing the pretreatment progesterone levels in our patients who had gone into labour with those that had not after treatment with PGE_2 showed no difference; these results suggest that progesterone has no significant influence upon the onset of labour.

A similar conflict over the possible role of prolactin in parturition exists. It has been postulated that prostaglandins may mediate the action of hypothalamic releasing factors in pituitary function (Vale *et al*, 1971). In support of this, Yue *et al* (1974) demonstrated a significant increase in serum prolactin levels during second trimester abortion following the intrauterine administration of prostaglandins. No such rise was observed in the present study in those patients who failed to go into labour following prostaglandin treatment. It may be that the dose of prostaglandin given was insufficient to provoke the rise or that an increase was not observed as the release of prolactin is already at maximum capacity at the time of parturition. In contrast, however, an association between the decline of plasma prolactin concentrations and the onset of labour has been reported although the significance of this is not well understood (De Gezelle *et al*, 1977). Our observation that there was no difference in circulating prolactin levels before treatment in those patients who went into labour following PGE_2 treatment and those who did not would indicate that prolactin does not play a major role in regulating the processes which initiate the onset of labour.

Our results suggest however that a more reliable and predictable method of inducing labour with vaginal prostaglandins might be achieved by either a pre-induction assessment of circulating oestradiol-17β levels and titration of the dose of prostaglandins given, or the administration of some oestradiol combined with prostaglandin in the viscous gel.

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INHIBITION OF PROSTAGLANDIN-INDUCED UTERINE ACTIVITY BY NIFEDIPINE

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Summary

The effects of the calcium antagonist, nifedipine, on uterine activity induced by prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$) and E_2 (PGE_2) were studied in women undergoing therapeutic midtrimester abortion, and in patients with a missed abortion in the 16th to 27th week of pregnancy. In the five subjects receiving intra-amniotic $PGF_{2\alpha}$ (25 to 40 mg) for midtrimester abortion, nifedipine (30 mg orally) decreased uterine activity from a mean of 372 to 203 Montevideo Units. The effect on the intensity of the contractions was pronounced; frequency and basal tone were little affected. In patients with missed abortion, uterine contractions were induced by extra-amniotic application of PGE_2 (0·5 to 1·5 mg) in a viscous gel. The activity was often more irregular than that in the women receiving intra-amniotic $PGF_{2\alpha}$. However, nifedipine (30 mg orally) had a marked inhibitory effect on the uterine contractions. It is concluded that nifedipine can be used for treatment of uterine hyperactivity induced by prostaglandins. Combined treatment with β_2-adrenoceptor stimulants might be considered.

PROSTAGLANDINS administered in different ways are widely used for interruption of pregnancy (Karim and Amy, 1976; Barden, 1977), for induction of labour after intrauterine fetal death (Lippert and Lüthi, 1978), and also for induction of labour at term (Thiery and Amy, 1976; Calder *et al*, 1977; Kennedy *et al*, 1978). As overstimulation of the uterus may be a problem (Craft and Youssefnejadian, 1974; Borten and Friedman, 1978), drugs that rapidly and effectively inhibit the uterine effects of exogenous prostaglandins would be of clinical importance. Previous investigations have shown that β-adrenoceptor stimulants such as orciprenaline, ritodrine, and terbutaline can inhibit prostaglandin-induced activity, both at mid-term (Lindmark *et al*, 1973; Andersson *et al*, 1975) and at term (Baillie and Jonathan, 1972; Scher and Baillie, 1972).

Calcium antagonists (Fleckenstein, 1977) effectively reduce prostaglandin-induced electrical and contractile activity in rat myometrium (Reiner and Marshall, 1976). Recently, it was demonstrated that one of these drugs, nifedipine, markedly inhibited uterine activity in normal women (Ulmsten *et al*, 1978) as well as in women with dysmenorrhoea (Andersson and Ulmsten, 1978).

In the present study, we have investigated the effects of nifedipine on uterine activity induced by prostaglandins $F_{2\alpha}$ and E_2 in women having a therapeutic abortion in the second trimester, and in women with a missed abortion in the 16th to 27th week of pregnancy.

METHODS

Two groups of patients were studied. The investigation was fully explained to each patient, and informed consent obtained.

Group I

Group I consisted of five healthy women, aged 19 to 41 years (mean 24 years) weighing 60 to 82 kg (mean 68 kg) admitted to the hospital for therapeutic abortion in the 16th to the 22nd week of pregnancy. Four were primigravidae and one multigravida.

After amniocentesis, a catheter for epidural anaesthesia (Portex 100/380/20, open with side holes) was inserted into the uterine cavity. Through this catheter, 25 to 40 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was instilled. A pressure transducer (Hewlett Packard 1280 B8) was connected to the catheter, and the diaphragm was set at the level of the symphysis. Intrauterine pressure was recorded on a cardiotocograph (Hewlett Packard 8030). Uterine activity was expressed in Montevideo Units and calculated for 10-minute periods. Maternal heart rate was monitored on the cardiotocograph by means of an ultrasonic flow detector (Hewlett Packard), and blood pressure was recorded by auscultation every five minutes.

Three to five hours after instillation of $PGF_{2\alpha}$, the intrauterine pressure was recorded continuously when labour-like contractions had been recorded for at least 30 minutes, the patients were given 30 mg of nifedipine orally, and uterine activity was recorded for at least two hours.

Analgesics were not given during the recordings. No patient received oxytocin.

Group II

Group II consisted of six women, aged 18 to 34 years (mean 29 years), weighing 48 to 62 kg (mean 55 kg) with a diagnosis of missed abortion in the 16th to 27th week of pregnancy. Two of them were primigravidae, four were multigravidae.

Uterine activity was induced by intracervical administration of 0·5 to 1·5 mg of PGE_2 suspended in a viscous gel; two patients had been pre-treated for three days with 1 mg of ethinyloestradiol.

In three of the patients, intrauterine pressure was recorded extra-amniotically by means of two microtransducers (Millar Instruments, Houston, Texas) enclosed in a teflon catheter 6 cm from each other. In three other patients, intrauterine pressure was recorded extra-amniotically by conventional open-ended catheters (Hewlett Packard). After amplification, the pressure signals from the transducers or the catheters were recorded by means of an Omniscriber WTR 281 (Watanabe, Japan) or a cardiotocograph (Hewlett Packard 8030). In all patients, the recording catheter was inserted through the cervix.

Within one hour after the administration of PGE_2 gel, intrauterine pressure was recorded continuously. When the patients experienced labour-like pain, and uterine activity had been recorded for at least 30 minutes, 30 mg of nifedipine was given orally. Uterine activity was recorded for another two hours. Maternal blood pressure and heart rate were recorded by auscultation at 10-minute intervals during the recordings.

RESULTS

Group I

In all patients, uterine activity decreased at 10 to 20 minutes after administration of nifedipine. The mean value before drug administration was 372 Montevideo Units. After, it decreased to a minimum of 203 Montevideo Units. The effect was most pronounced after 40 minutes, and in no patient did the uterine activity return to the starting level during up to 300 minutes of recording.

The decrease in uterine activity after nifedipine administration was caused by a marked decrease in the intensity of the contractions (Fig. 1). From a mean value of 46 mm Hg, there was a decrease to a minimum of 20 mm Hg.

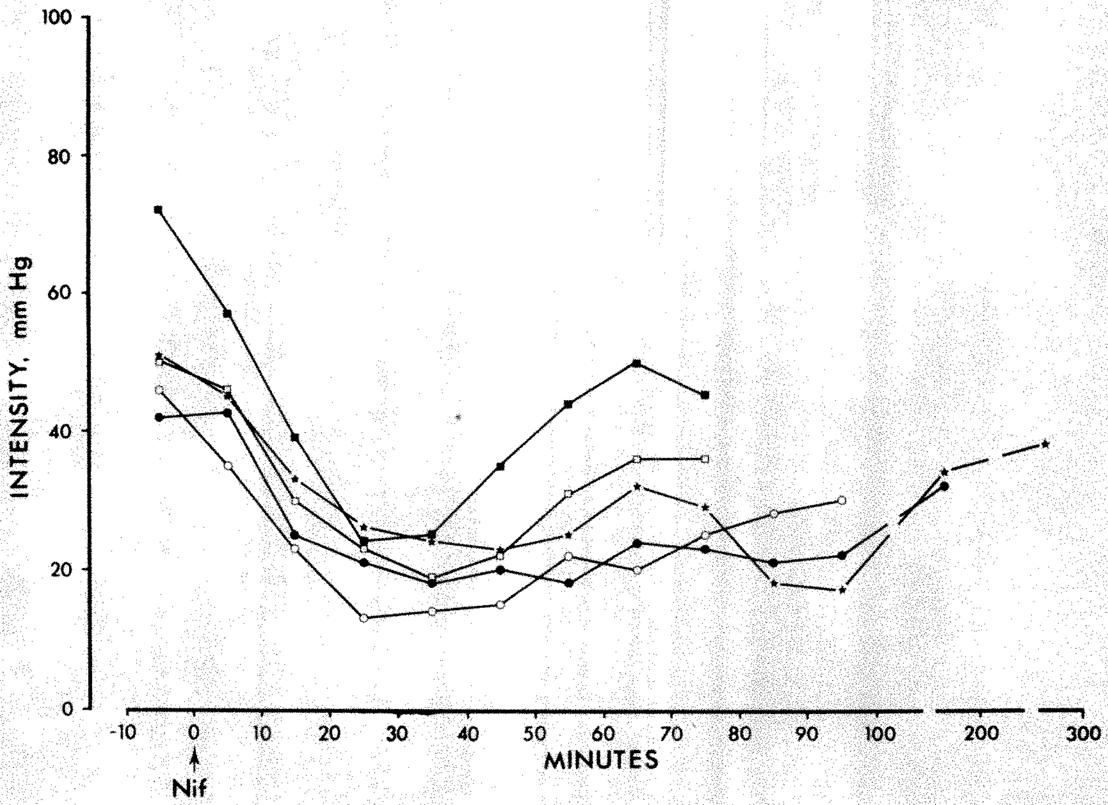

FIG. 1

Effect of 30 mg of nifedipine, given orally, on the intensity of the uterine contractions induced by intra-amniotic administration of $PGF_{2\alpha}$ in five patients.

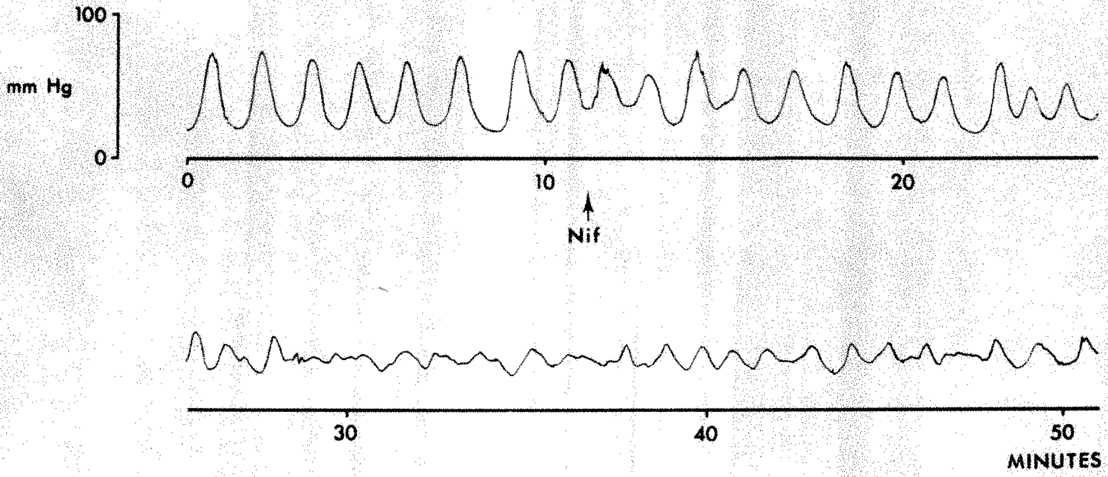

FIG. 2

Recording of intrauterine pressure (open-end catheter) in a patient given prostaglandin $F_2\alpha$ (40 mg) intra-amniotically for therapeutic abortion (21st gestational week). Nifedipine (Nif) 30 mg reduced the amplitude of contraction, but had little effect on frequency and basal tone.

A slight increase in the number of contractions from 7·2 to 8·0 in 10 minutes, was recorded, and a small increase in basal tone was observed during the first hour of drug administration (Fig. 2).

After taking nifedipine, the patients felt relief of the labour-like pains induced by the prostaglandin. There was an increase in maternal heart rate by 5 to 20 beats/minute, but no consistent changes in maternal blood pressure were recorded. All patients had transient facial flushing, occurring 10 to 20 minutes after drug intake, and some reported a transient headache. When the recordings were finished, a second injection of intra-amniotic $PGF_{2\alpha}$, 15 to 25 mg, was given. The mean time to abortion was 20 hours (range 9 to 45 hours).

Group II

The uterine activity induced by the PGE_2-gel was more varying than that after intra-amniotic $PGF_{2\alpha}$, making quantitation difficult. However, in all patients nifedipine reduced (4 patients) or abolished (2 patients) the uterine contractions. Intensity of contraction was reduced in all women, whereas basal tone decreased in 4 and increased in 2. Frequency was slightly increased in the 4 women in whom the activity was not abolished. Simultaneously with the reduction in uterine activity, the labour-like pain experienced by the patients was markedly reduced or abolished. As in Group I, all patients had facial flushing and two of them experienced a transient headache. The duration of the inhibitory effect of nifedipine was up to 5 hours. A moderate increase in heart rate (5 to 20 beats/minute) was observed after drug intake; there was no consistent change in blood pressure. Abortion occurred within 8 to 14 hours after administration of the PGE_2 gel. All patients had an evacuation of the uterus and none had complications.

DISCUSSION

The way in which prostaglandins act on the myometrium has not been established. The rat uterus can be activated either by an inward calcium current, or by release of calcium from intracellular stores (Mironneau, 1973). Reiner and Marshall (1976) showed in rat myometrium that the response to maximal doses of $PGF_{2\alpha}$ consisted of an increase in tone with superimposed contractions. Two types of membrane phenomena corresponded to these changes, namely slow depolarizations and spikes. The spike activity and the contractions could be blocked by calcium antagonists, or removal of calcium from the extracellular medium, and were attributed to calcium influx. Slow depolarization and tone could be induced by $PGF_{2\alpha}$ in calcium-free solution, and in the presence of calcium antagonists. These effects were assumed to be caused by release of calcium from intracellular stores. It has been shown in several studies that prostaglandins can release calcium from isolated sarcoplasmic reticulum (Carsten, 1973) and mitochondria (Malmström and Carofoli, 1975), and an effect primarily on intracellular calcium stores was suggested by Grosset and Mironneau (1977). However the mechanism that predominates may depend on the dose of prostaglandin or on the species being investigated.

The present results suggest that with the doses of prostaglandins used, inflow of calcium from the extracellular medium might be an important way of activating the human uterus, as nifedipine, which is believed to selectively inhibit calcium inflow, effectively reduced uterine activity. This action might be clinically useful for inhibition of prostaglandin-induced hyperstimulation of the uterus.

In a previous study (Andersson *et al*, 1975), the effect of the selective β_2-adrenoceptor stimulant, terbutaline, was investigated on uterine activity induced by $PGF_{2\alpha}$ or hypertonic saline in patients undergoing mid-trimester abortion. In the prostaglandin group, terbutaline reduced uterine activity from 393 to 214 Montevideo Units. The reduction was primarily caused by a decrease in the frequency of contraction, but also basal tone was diminished. Intensity of contraction, on the other hand, was little affected. β-Adrenoceptor stimulants are believed to relax the uterus by stimulating calcium uptake in intracellular stores, thereby reducing the amount of free calcium in the cytoplasm available for contraction. It was therefore interesting to note that in the present study, in which the patients of Group I and the investigation technique were similar to those in the study by Andersson *et al* (1975), nifedipine caused approximately the same inhibition of uterine activity as terbutaline (from 373 to 203 Montevideo Units). The effect of nifedipine was almost solely caused by a decrease in the intensity of contraction, whereas frequency was little affected, and basal tone even increased. This is in agreement with the view that nifedipine has a main site of action in or at the membrane, preventing the inflow of calcium caused by $PGF_{2\alpha}$, and that it has less effect on the intracellular actions of $PGF_{2\alpha}$ which maintain or increase basal tone.

Thus, both β-adrenoceptor stimulants and calcium antagonists seem to be useful for control of prostaglandin-induced uterine hyperactivity. As none of the agents can be expected to be effective in all cases, and because they decrease uterine activity by different mechanisms, a combination of the two kinds of drug might be effective.

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THE RELATION BETWEEN PLASMA UNCONJUGATED OESTRIOL AND CYSTINE AMINOPEPTIDASE CONCENTRATIONS AND UTERINE CONTRACTIONS

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Summary

The relation between maternal plasma unconjugated oestriol (E_3) concentrations and uterine activity was investigated by very frequent, carefully timed determinations of E_3 during synchronously recorded uterine contractions in six normal subjects. There was an initial rise (10 to 50 per cent above the mean) in E_3 levels reaching a peak coincident with the contraction peak (range 20 seconds before to 15 seconds after) followed by a fall of similar magnitude below the mean reaching a nadir about 80 seconds (range 60 to 105 seconds) after the contraction peak. Although the pattern of E_3 fluctuation was most marked in labour the overall degree of variation was no greater than at other times. Cystine aminopeptidase (CAP) concentrations in the same serum samples showed no significant correlation with uterine contractions. The reasons for, and practical applications of, the E_3 results are discussed.

IN earlier studies on plasma unconjugated oestriol (E_3) in individual women during late normal pregnancy we found that concentrations fluctuated to about the same degree at intervals ranging from a day to only a few minutes (median coefficients of variation (CV) were 15 per cent and 12 per cent respectively). Although there was an overall increase of about 15 per cent in E_3 levels at night, the fluctuation in individuals seemed otherwise to occur randomly (Hull *et al*, 1978). In an initial attempt to discover by more frequent sampling, whether there were really any pattern to the fluctuation, we observed intermittent reductions in E_3 concentrations, possibly due to uterine contractions reducing intervillous blood flow. We therefore embarked on the present study, taking very frequent blood samples during synchronously recorded uterine contractions.

In the light of the E_3 results obtained, we also determined plasma cystine aminopeptidase (CAP) because it is a placental product that is much more slowly cleared than unconjugated E_3 (the respective half-lives in blood being about 2 days and 15 minutes) and metabolic clearance rate must affect the degree of fluctuation of the circulating levels of any substance.

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Subjects and Methods

Six healthy volunteers were studied, those in labour having given consent before its onset. Their clinical details and the conditions of study are given in Table I. Five patients were at term, four of these in labour, and the sixth was at 26 weeks, not in labour. They all had normal pregnancies. Patient No. 1 was supine and all others were studied in the lateral position. The two studies done on subject No. 3 were analysed separately.

A 2 ml volume of blood was rapidly collected every 15 to 30 seconds over 20 minutes (in subject No. 3, divided into two separate 10-minute periods) through an indwelling cannula in a forearm vein into a heparinized syringe. The intervals were accurately timed, and kept constant in any individual. Uterine contractions were recorded simultaneously by external tocography. The uterine 'pressure' at the time of each sample was ascribed a nominal value derived from the measured height of the record. The fetal heart rates were also recorded continuously and were normal.

The separated plasma samples were stored at $-20°C$ until assayed. Ether extracts of 0·1 ml plasma were radioimmunoassayed using an antiserum to E_3-6-oxo-carboxymethyl oxime-BSA, and using $[^3H]$ E_3 as label for scintillation counting to determine unbound steroid after precipitation of bound steroid with ammonium sulphate. The samples were assayed in duplicate and all from any individual subject were assayed in the same batch. The intra-assay coefficient of variation (CV) calculated from duplicates in individuals was 4·6 to 7·3 per cent (median 5·9 per cent) (Table I).

Plasma CAP was determined by the automated kinetic method of Tovey *et al* (1973), in which the rate of conversion of substrate is measured by colorimetry, modified by the introduction of 0·03 per cent BRIJ 35 into the

Table I

Clinical details of subjects studied and plasma unconjugated E_3 results (1 μg E_3 = 3·467 nmol). The fluctuation of E_3 values in any individual is given by the coefficient of variation of the values. The assay error is given by the coefficient of variation calculated from duplicates

				Conditions when studied					Plasma E_3		
Subject No.	Age (years)	No. of previous pregnancies of more than 28 weeks	Birth weight (kg)	Gestation (weeks)	Labour and onset	Membranes	Drugs	Contraction intervals (minutes)	Mean (μg/l)	Fluctuation (per cent)	Assay error (per cent)
1	33	3	3·57	40	Labour, induced	Intact	Oxytocin	2·4	16·0	13·6	5·8
2	24	0	3·46	42	Labour, induced	Ruptured	Oxotycin, Pethidine	2·9	12·0	6·5	5·6
3(a)	28	0	2·80*	40	(a) Labour, spontaneous	Intact	None	3·3	4·9	12·8	4·5
(b)					(b) 1½ hours later	Intact	None	3·0	4·2	12·8	7·3
4	28	0	3·80	41	Labour, spontaneous	Intact	None	7·0	11·6	15·7	4·6
5	27	1	4·14	40	Not in labour	Intact	None	8·5	12·2	8·3	7·3
6	36	2	3·75	26	Not in labour	Intact	None	>20	3·0	10·8	6·7

* Below 10th centile (Thomson *et al*, 1968)

buffer to suppress the turbidity that occurs with plasma. All samples from any individual were assayed in the same batch. The within-assay CV of replicate controls in the same batches as the samples was 1 to 2 per cent.

Relation between the fluctuations in E$_3$, CAP and uterine pressure values obtained, and the timing of the relationships, were tested by computerized time series cross correlation (TSCC) analysis of paired sets of data (Ellis *et al*, 1978) using Pearson's correlation coefficient (r). TSCC involves moving one set of data over the other step-wise, each step being from one observation to the next, and calculating the correlation coefficient at each step. In this way any positive or negative correlation between the data sets could be plotted against the time-shift between them, and thus any variation in the correlation represented as wave-form related to time. The time-relationship was taken to be best at the time of the peak r-value of each wave of correlation. Correlations were accepted as significant if probability was 0·02 or less (two-tailed test).

RESULTS

The results of the serum E$_3$ and CAP determinations are shown plotted above the simultaneously recorded uterine tocograph for three patients (No. 1, 2 and 4) in Figures 1 to 3. The results of TSCC analysis of the E$_3$ data against uterine pressure in *all* subjects are given in Table II. In the figures, the E$_3$ and CAP values have all been plotted to the same scale by expressing the results as per cent of the overall mean in any individual. The actual mean values for E$_3$ are given in Table I, together with the overall degree of fluctuation (CV) in individuals, which ranged from 6·5 to 15·7 per cent (median 12·8 per cent). The limits of fluctuation in individuals varied between an increase at the start of uterine contractions of 10 to 50 per cent above the mean, and a subsequent fall of 10 to 40 per cent below the mean. The overall degree of fluctuation of CAP values in individuals was only 1·1 to 1·5 per cent, no greater than the assay error.

From the Figures 1 to 3 it is clear that the serum unconjugated E$_3$ concentrations fluctuated with uterine contractions but there was no appreciable fluctuation in CAP. In subject No. 1

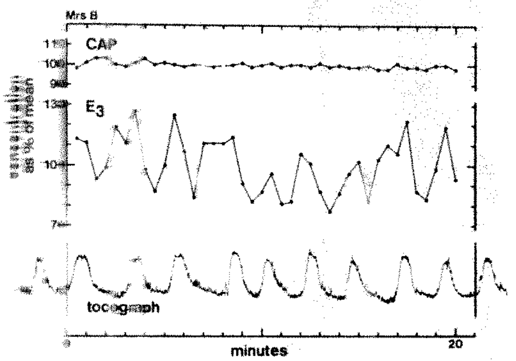

FIG. 1

Plasma unconjugated oestriol (E$_3$) and cystine aminopeptidase (CAP) concentrations every 30 seconds, expressed as per cent of the overall mean, and the simultaneous external uterine tocograph in patient No. 1 who was in labour at term gestation (for her details see (Table I).

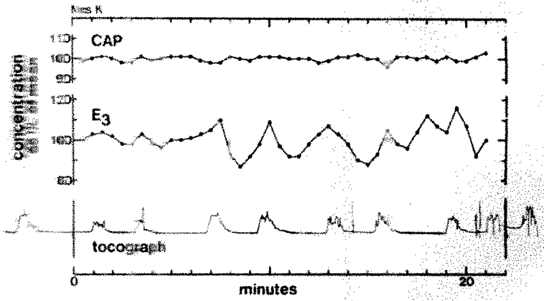

FIG. 2

Findings in patient No. 2 who was in labour at term, plotted as in Figure 1. Blood samples were taken every 30 seconds.

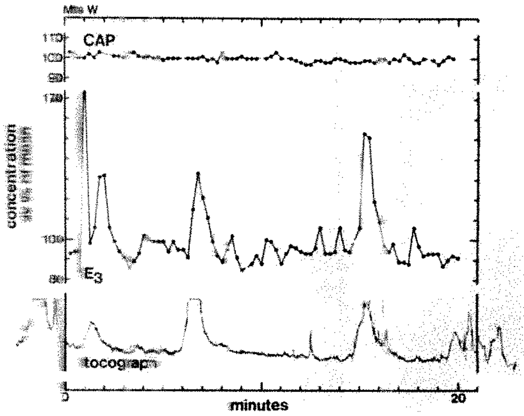

FIG. 3

Findings in patient No. 4 who was in labour at term, plotted as in Figure 1. Blood samples were taken every 15 seconds.

TABLE II

The peak features of the waves of correlation (coefficient r) between serum unconjugated E_3 and uterine pressure found by time series cross correlation: the peak r-values and their timing. The results are given only for waves with significant r values ($p \leq 0{\cdot}02$) occurring within three minutes either way of the observed time (zero). The sign of the time-shift is negative if the uterine pressure series has been retarded with respect to the E_3 series, indicating that the particular E_3 fluctuation is in advance of uterine contraction, and vice versa. The sign of the r-value being positive indicates a rise in E_3 associated with uterine contractions, and negative indicates a fall. The number of pairs of data in each calculation is given by n.

Subject No.	n	Time-shift (seconds)	r	Time-shift (seconds)	r	Time-shift (seconds)	r
1	40	0	+0·510	+60	−0·543	+120	+0·494
2	42	0	+0·464	+60	−0·623		
3(a)	31	+15	+0·481				
(b)	39						
4	78	+15	+0·578				
5	41	−20	+0·368	+100	−0·472		
6	71	0	+0·445	+105	−0·361		

(Fig. 1) the contractions were so frequent that it was difficult to recognise a baseline for E_3, and therefore difficult to decide whether the contractions were associated with rises or falls in E_3 values, or both. However it looked as if the best correlation was between the nadirs of the E_3 fluctuations and the contraction peaks, which occurred about one minute before the E_3 nadirs. In subject No. 2 (Fig. 2) an E_3 baseline was detectable in the longer intervals between some contractions and it looked as if there was a rise in E_3 values coincident with contraction peaks, followed by a fall to a nadir one minute later. In subject No. 4 (Fig. 3), in whom uterine contractions were occurring less frequently than the previous subjects, there was a consistent, marked rise in E_3 almost coincident with the contraction peaks, followed by only a small fall below basal values.

In general, the variation of E_3 with uterine contractions was more obvious in those subjects in labour, as in the cases illustrated in Figures 1 to 3, than in those who were not in labour, but the overall degree of fluctuation or coefficient of variation (CV) was not much greater (Table I).

TSCC analysis of E_3 values and uterine pressure revealed significant ($p \leq 0{\cdot}02$) associations between E_3 fluctuations and uterine contractions as set out in Table II. Correlations are shown only within three minutes either way of the observed time because beyond these limits there is unlikely to be any true relationship and in any case repeated waves of correlation would be expected whenever fluctuations occurred with a regular cycle. In fact this latter finding only occurred in subject No. 1 for a few extra cycles, and in subject No. 2 for one extra cycle. In subject No. 3 the relative lack of significant correlations reflects the inconstancy of the changes observed with different contractions, i.e. only an elevation in E_3 with some contractions and only a reduction with others, although some individual changes were clearly significant in their degree (demonstrable by a lack of fit test for an analysis of variance model) and their timing occurred as in other patients.

The results of analysis in Table II have been collated diagrammatically in Figure 4. Thus there was (1) a wave of positive correlation in six patients suggesting a rise in E_3 levels occurring with a peak between 20 seconds before and 15 seconds after (median zero or coincident with) the contraction peak; (2) a wave of negative correlation in four patients suggesting a fall in E_3 levels occurring with a nadir 60 to 105 seconds (median 80 seconds) after the contraction peak; and (3) in one patient, No. 1, there was a second positive correlation wave

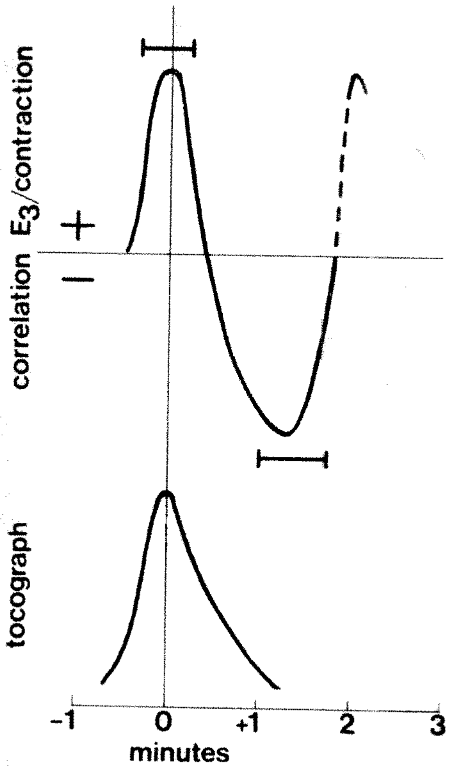

FIG. 4

Schematic representation of the results given in Table II of time series cross correlation between serum unconjugated oestriol (E_3) concentrations and uterine pressure. Each horizontal bar represents the range of times, related to the contraction peak, at which the peak correlations (positive and negative) were found.

suggesting a rise in E_3 occurring 120 seconds after the contraction peak. Although this may represent a return to baseline after the fall in E_3 it seems more likely to indicate a rise associated with the following contraction since the intervals between contractions were so short.

TSCC analysis of CAP values versus uterine pressure or E_3 revealed much fewer significant waves of correlation and these had a random pattern, suggesting that they were spurious, this interpretation being supported by the appearance of the data plotted in Figures 1 to 3.

DISCUSSION

The results of this study revealed a fluctuation of peripheral plasma unconjugated E_3 concentrations apparently associated with uterine contractions and occurring even in women not in labour. There was also a lesser, apparently random variation in E_3 concentrations which was probably partly due to assay error and partly random variations in production and metabolic clearance rates. The overall variation in E_3 levels (CV, see Table I) was no greater in strong labour (subjects No. 1 and 2) than at other times (Hull *et al*, 1978). Neither the dorsal position (subject No. 1) nor amniotomy (subject No. 2) seemed to alter the E_3 pattern.

The exact relationship between E_3 levels and uterine contraction depends on the circulation time between uterine and forearm veins. Lippert *et al* (1973) found that isotopic tracer first reached the placenta *from* a forearm vein in about 30 to 60 seconds and the return journey is likely to take a similar time. If such is the delay, our study indicates that the rise and fall in peripheral venous E_3 concentrations are respectively related to the onset and end of contractions. This pattern fits in well with the generally accepted view (Sureau, 1974; Assali *et al*, 1978) that uterine venous blood is expelled at the onset of a contraction with a resultant increase in central venous pressure and cardiac output, arterial flow continuing after the veins are occluded but later also ceasing as intra-amniotic and intramyometrial pressure rises further.

Assuming a relatively constant clearance of unconjugated E_3, variations in circulating E_3 levels must be determined by changes in blood production rate. Our findings suggest that the production rate depends on intervillous blood flow and it is quite possible (but not yet shown) that the concentration of E_3 in intervillous blood is fairly constant. This idea is supported by Townsly's *in vitro* studies which showed that the conversion rate of oestrogen precursors by the trophoblast depends mainly on the rate at which earlier precursors are supplied and the product removed (Townsley *et al*, 1973; Townsley, 1975).

While the rise in peripheral venous E_3 levels induced by the onset of uterine contraction is easily explained by the rapid injection into the circulation of a bolus of placental blood containing a high concentration of E_3, the rapid fall in E_3 is probably due to dilution by hepatic venous blood, which is probably relatively free of unconjugated E_3 (as for other diffusible steroids; Tait and Burstein, 1964).

The possibility of measuring placental blood flow using a specific placental marker like E_3 at first appears attractive. However, peripheral E_3 levels reflect not only changes in placental blood flow but also other changes such as in hepatic venous return. Thus peripheral E_3 levels cannot be expected to provide more than a *relative* index of changes in placental blood flow. Random fluctuations and changes induced by uterine contractions could be integrated by continuous rather than intermittent blood sampling and the effect of drugs or posture on placental blood could thus be studied without using isotopic tracers (e.g. Guikovaty *et al*, 1975; Gant *et al*, 1976). We are currently investigating the value of E_3 assays used in this way.

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COMPUTER ANALYSIS OF FETAL HEART RATE VARIATION DURING NORMAL PREGNANCY

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Summary

Ninety-seven one-hour recordings of the abdominal fetal electrocardiogram (ECG) were made from 59 normal patients between 21 and 41 weeks of gestation. The heart intervals, measured between successive R-waves, were analysed by computer. The signal-to-noise ratio of the fetal ECG limited the precision of the interval measurements to approximately one millisecond. The characteristics of the baseline heart rate changed significantly as gestation advanced, the mean R-R interval, the standard deviation of the intervals and the standard deviation of the interval differences all increasing with gestation ($p < 0{\cdot}001$). In later gestation the baseline heart rate during periods of fetal rest differed significantly from that during periods of fetal activity; during rest the mean R-R interval was greater ($p < 0{\cdot}001$) and the standard deviations of the intervals and interval differences were smaller ($p < 0{\cdot}001$). Examination of the coefficient of variation of the heart intervals gave a result which contradicted the significance of this measurement as an index of fetal welfare as proposed by Curran and MacGregor (1970).

Computer analysis of the fetal heart rate (FHR) can provide an objective means of condensing the large quantity of information which results from continuous monitoring of the fetus. In particular, a more accurate analysis of cardiac beat-to-beat variation can be obtained compared with that provided by conventional FHR monitors. The relatively slow recording speeds of the latter, combined with the small scales allowed for the display of the heart rate, make it difficult to distinguish the changes in rate from beat to beat. Furthermore the scales, being linear to frequency, compress the changes in heart interval at the lower rates and expand them at higher rates.

Reliable observations of heart interval can only be made by measuring the time-periods between successive R-waves of the fetal ECG; signals derived from phonocardiography or ultrasound are too variable to provide an accurate measurement of each cardiac cycle. From approximately 20 weeks gestation, the FHR can often be derived from the fetal ECG detected on the maternal abdomen although this may be difficult between 28 and 34 weeks due to the low amplitude of the signals at this time (Larks, 1961; Bolte, 1969). When the abdominal fetal ECG is used to record the FHR, the maternal ECG, which is also detected, must be removed during the processing of the signals. The 'blanking' technique often used for this purpose also removes fetal signals when they coincide with maternal complexes so that fetal pulses have to be substituted. Since this affects 15 to 20 per cent of the fetal signals, a loss of 30 to 40 per cent of the genuine beat-to-beat information is inevitable. To avoid this problem in the present study, a system of subtraction was used in which the maternal complexes appearing in the abdominal record were subtracted from themselves and, with the aid of an analogue store, also from the combined feto-maternal complexes, thus preserving all the fetal signals (Wheeler *et al*, 1978*b*).

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Computer analyses of the FHR can be designed to describe both its long-term variation (already apparent in the cardiotachogram) and its short-term variation (from beat to beat) which is seldom visible in the cardiotachogram. Several mathematical expressions for these features have been proposed, notably by de Haan *et al* (1971) and Yeh *et al* (1973); these indices have recently been discussed by Laros *et al* (1977). In the main program developed for this study, the R–R intervals were measured in milliseconds (ms) and the following parameters were calculated: the mean heart interval, the standard deviation of the intervals, the coefficient of variation of the intervals and the standard deviation of the interval differences. The effects of fetal maturity and of fetal state (resting or active) upon these parameters have been investigated. The proposal of Curran and MacGregor (1970) that the coefficient of variation of the heart intervals is predictive of fetal outcome has also been examined. The data were obtained from patients with normal pregnancies and have been reviewed previously in the form of cardiotachograms (Wheeler and Murrills, 1978).

In a recent investigation of heart rate variation in fetal lambs, Dalton *et al* (1977) found that 46 per cent of the interval differences were of less than one millisecond. They also demonstrated multiple rhythms in the heart rate which caused the distribution of the heart intervals to be non-Gaussian and their standard deviation to increase with the period of observation. These fundamental points are considered at the outset of this paper.

PATIENTS AND METHODS

Ninety-seven recordings of the fetal ECG were obtained from 59 normal patients between 21 and 41 weeks gestation. The fetal ECG was recorded from the surface of the maternal abdomen using lead electrodes placed at the fundus and symphysis pubis. The signals were stored on magnetic tape using Philips cassette recorders (models N2205, N2225 and EL3302P). Each recording lasted for one hour. On subsequent replay the signals were processed using a subtraction technique to remove the maternal ECG (Wheeler *et al*, 1978*b*). The processed fetal signals were then detected as they crossed an automatically adjusted threshold, a trigger pulse being generated by the first zero-crossing to follow each threshold detection. The trigger pulses so obtained were again stored on magnetic tape and at the same time used to produce a cardiotachogram (Hewlett Packard recorder type 8025A). The cardiotachograms have been reviewed previously and further details of the patients are given in the same paper (Wheeler and Murrills, 1978).

Of the original 97 recordings, five were excluded from the computer analysis because the cardiotachograms showed either a progressive tachycardia or a cardiac arrhythmia. In the remaining 92 recordings, the fetal R–R intervals were analysed by replaying the trigger pulses into a PDP 12 computer (Digital Equipment Corporation) programmed to measure the intervals between successive trigger pulses to a resolution of one millisecond. Artifactual intervals could be identified by reference to the cardiotachograms (Fig. 1), and before a recording was entered into the computer, limits were set to

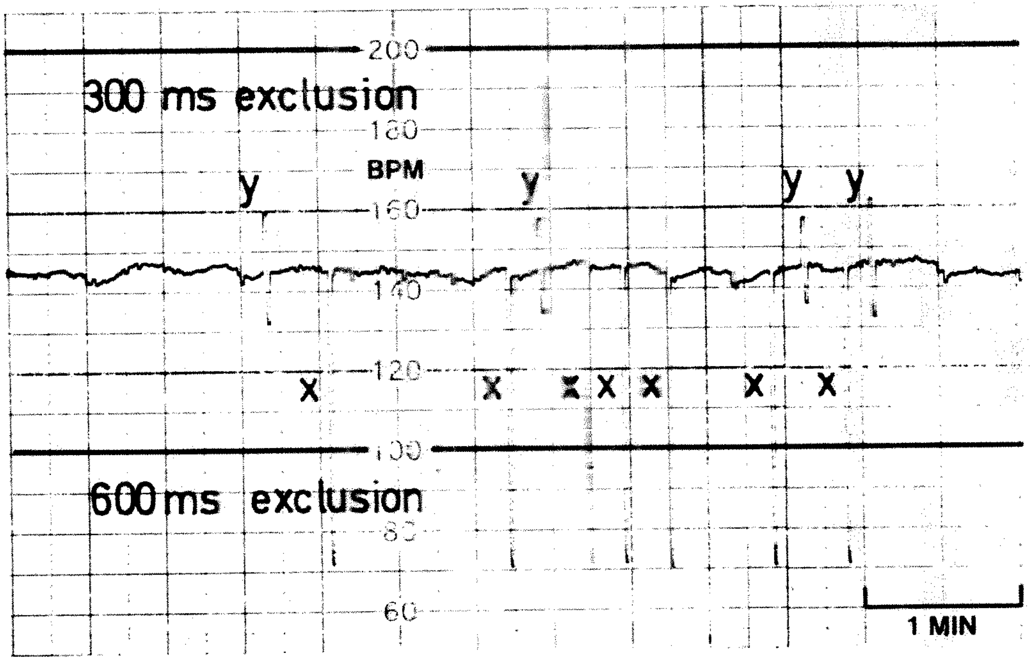

FIG. 1

Computer exclusion of artifacts illustrated in a cardiotachogram made on the Hewlett-Packard 8025A recorder at 2 cm/minute. Intervals of less than 300 ms are excluded during the processing of the fetal ECG signals. The large artifacts (x), due to missing fetal trigger pulses, are excluded by a maximum limit set to 600 ms in this example; the following interval is accepted. The smaller errors (y), due to mistriggering, are excluded if they represent a change which exceeds a defined percentage of the previously accepted interval; the succeeding interval is also artifactual and excluded by definition.

exclude these. Due to the incorporation in the earlier signal processing of a paralysis period after each fetal trigger pulse, intervals of less than 300 ms had already been excluded. Two further exclusions, applied in order, were made by the program (Fig. 1). The first removed any interval which exceeded a specified duration (usually due to a missing fetal trigger pulse). The second limit defined the maximum percentage by which any heart interval could differ from its accepted predecessor. An interval excluded on this criterion was normally the result of mistriggering due to noise or distortion of the fetal complex and in this circumstance the following interval was also incorrect and excluded.

For each sample of 256 successive intervals the computer measured the following variables: the mean heart interval, the standard deviation of the intervals, the coefficient of variation of the intervals and the standard deviation of the interval differences. In a number of recordings measurements were also made of the 'differential index' (Yeh *et al*, 1973) and the mean absolute interval difference. Reference to the cardiotachogram enabled each sample to be placed into one of three categories: baseline heart rate (representing about 80 per cent of any recording), accelerations, or decelerations. These distinctions were made because accelerations and decelerations caused considerable changes in the measurements and probably represented altered physiological conditions. However, to permit comparison with other published work, a number of measurements were made over longer epochs without distinguishing between the data categories.

MEASUREMENT PRECISION

Three sources of error were considered pertinent to the measurements: the signal-to-noise ratio of the fetal ECG signals, the variations in speed of the tape-recorders and the resolution of the computer clock. Of these, the signal-to-noise ratio set a fundamental limit to the accuracy with which the heart intervals could be measured. Each source of error was investigated and its effect on the data assessed.

1. *Signal-to-noise ratio*

The trigger pulse which defined each fetal heart beat was generated by a negative-going zero-crossing of the processed fetal QRS complex. The exact moment of this occurrence could be perturbed by noise on the signal, the magnitude of the effect depending on the amplitude of the noise and the slope of the signal. Errors in timing of the trigger pulses naturally gave rise to errors in the R–R interval and interval difference measurements. The variable nature of the effect necessitated an on-line assessment of the problem (Fig. 2). This was achieved by generating artificial fetal ECG signals free from both noise and variation in rate and having a downslope at the trigger point equal to that of the current real ECG signals. To this ideal simulated fetal ECG was added the noise from the real signals. Trigger pulses, derived from the artificial signals thus produced, were then fed to the computer which measured the inter-pulse intervals (nominally 400 ms) to a resolution of 100 μs. The mean interval, the standard deviation of the intervals and the standard deviation of the interval differences were calculated. A histogram of the interval differences (resolved to 100 μs) was also obtained.

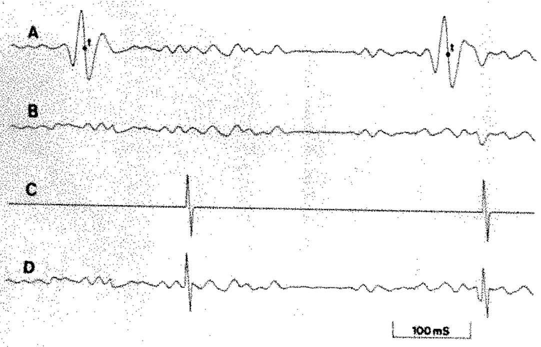

FIG. 2

Effect of signal-to-noise ratio on timing errors. A: processed fetal ECG signal. B: noise extracted from trace A. C: noise-free artificial signals with down-slope equal to that at trigger points (t) in trace A; constant inter-pulse interval of 400 ms. D: summation of traces B and C; noise disturbs trigger points and causes apparent variation in inter-pulse interval statistically equal to that due to noise in trace A.

For the recordings analysed in this study the average value of the standard deviation of intervals *due to noise alone* (mean ± SD) was 0·9 ± 0·4 ms; the corresponding value for the standard deviation of the interval differences was 1·4 ± 0·5 ms. Neither figure changed significantly with gestation. Before correction by root-square subtraction, the data presented in this paper ranged from 4 ms to 27 ms for the standard deviation of the intervals and from 2 ms to 13 ms for the standard deviation of the interval differences, so that the contribution to these figures from variation due to noise was only significant at the lower ends of the ranges. This resulted from the root-sum-of-squares mode of combination of the standard deviation due to physiological variation with that due to noise.

In the case of the cumulative distribution of the interval differences (Fig. 3) a more elaborate correction procedure, that of constrained optimization deconvolution (Twomey, 1965), was

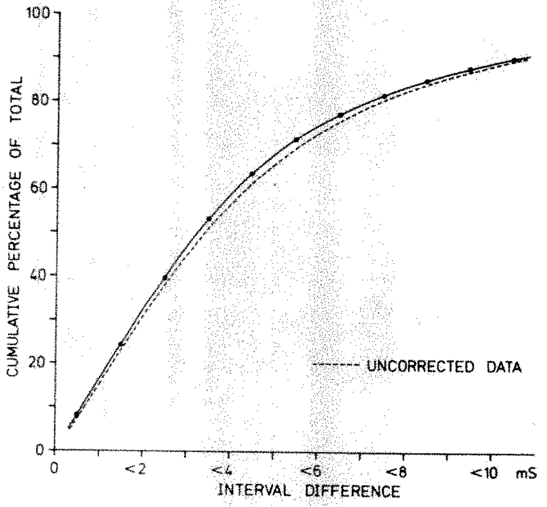

FIG. 3

Cumulative distribution of 19 193 R–R interval differences obtained from five patients over a period of 30 minutes in each case. The fetal trigger pulses were fed to the computer on-line and the intervals measured to a resolution of 100 μs. The data were deconvoluted to correct for errors due to noise on the fetal ECG. The average heart interval was 441 ms.

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used to ensure accuracy of the data even at the 1 ms level; however, the difference between the corrected and uncorrected distributions was small.

2. *Tape recorders*

Errors could arise from both long and short term discrepancies in the running speeds of the tape recorders. Relative differences in mean speed between machines could cause errors in the values for mean heart interval, whilst speed fluctuations could cause erroneous increases in the measurements of heart rate variation. Tape recorder performance was monitored throughout the study and mean running speeds were kept within one per cent of nominal value. Short term speed fluctuations were of the order of 1 per cent peak-to-peak for each machine. To assess the significance of these errors, several tests were made in which pulses at known and stable frequencies within the physiological range were subjected to a tape-recording process that simulated the original handling of the data. The reproduced pulse-trains were analysed by the computer working to 100 μs resolution. The greatest resulting error in mean interval was 1·5 per cent, whilst the standard deviations of the intervals and interval differences, *due to tape recording alone*, were respectively of the order of 2 ms and 0·7 ms. Again, due to root-sum-of-squares combination, only the lowest measurements of heart rate variation were significantly affected. A further test in which fetal heart rate data was analysed at 100 μs resolution, both before and after tape recording, gave results consistent with the above figures.

3. *Computer clock resolution*

Most of the data in this study were analysed at a computer clock resolution of 1 ms; this effectively introduced a small amount of artifactual heart rate variation. A mathematical analysis of the problem showed that the standard deviations of the intervals and interval differences *due to this effect alone* were respectively 0·6 ms and 1·2 ms, again only affecting the lowest values of the observed data.

The standard deviations of the intervals and interval differences due to combination of the three sources of error were 2·3 ms and 1·9 ms respectively. In the presentation of the results corrections for the relevant effects have been made where necessary.

RESULTS

Distribution of R–R interval differences in the human fetus

In five normal patients between 36 and 40 weeks gestation, fetal trigger pulses, derived from the processed abdominal fetal ECG, were fed directly into the computer to produce an on-line analysis of the R–R intervals and interval differences. Thirty minutes of data were obtained from each patient, giving a total of nearly 20 000 heart intervals which were measured to a resolution of 100 μs. The cumulative distribution of the interval differences (Fig. 3) showed that 16 per cent of the differences were of less than 1 ms. The mean heart interval for the five patients was 441 ms (136 beats/minute).

Effect of observation time on the standard deviation of the R–R intervals

In five recordings of the fetal ECG, each made from a different patient for a period of one hour, the standard deviation of the R–R intervals was measured over epochs of 15, 30,

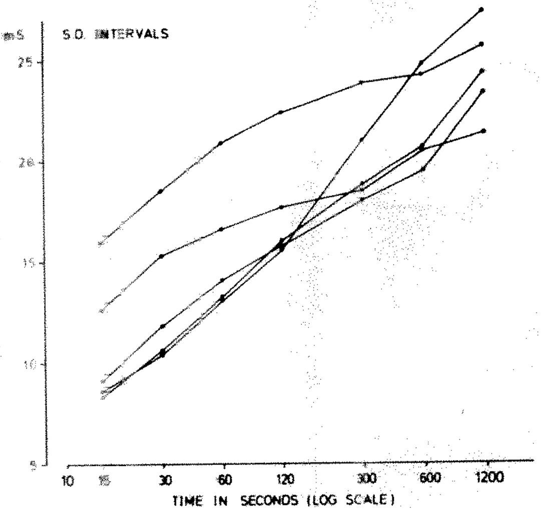


FIG. 4

The relationship between the standard deviation of the R–R intervals and observational epoch. Five different recordings, each lasting one hour, have been analysed.

60, 120, 300, 600 and 1,200 seconds. The values obtained over each epoch were averaged for each patient and plotted on a logarithmic time scale (Fig. 4). The standard deviation increased in an approximately linear fashion with the logarithm of observational epoch.

Effect of gestational age upon the mean and standard deviation of the intervals and standard deviation of the interval differences

Only periods of baseline heart rate were included in this part of the analysis, the data from all the recordings being divided into three groups of seven successive weeks gestation. The values for the standard deviation of the intervals and standard deviation of the interval differences measured over epochs of 256 intervals are shown in Figure 5. The measurements were also compared statistically by dividing the recordings into two groups of ten successive weeks gestation: 21 to 30 weeks and 31 to 40 weeks (Table I). If more than one recording had been made from a given patient in either group then only one record was included, this being selected using a table of random numbers. There was a significant increase ($p < 0{\cdot}001$) in the mean interval, the standard deviation of the intervals and standard deviation of the interval differences as gestation advanced.

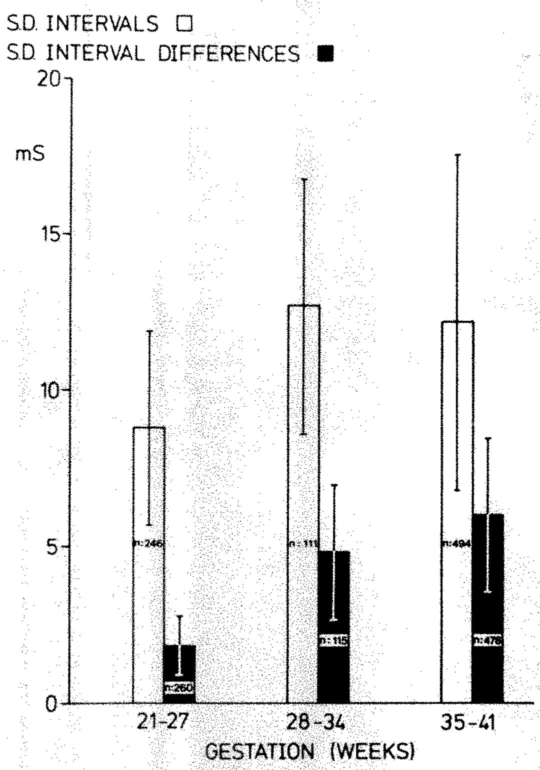


FIG. 5

Standard deviations of the R–R intervals and R–R interval differences during baseline heart rate related to gestation. The number of 256-interval epochs analysed to give mean ± SD is stated in each case.

TABLE I

Comparison of data from two periods of gestation: 21 to 30 weeks and 31 to 40 weeks

	Gestational age (weeks)		
	21 to 30 (21 recordings) Mean ± SD	31 to 40 (36 recordings) Mean ± SD	Significance*
Maternal age (years)	23·9 ± 4·3	25·6 ± 4·6	p > 0·1
Fetal maturity at delivery (weeks)	39·6 ± 1·5	39·9 ± 1·2	p > 0·4
Infant birth weight (g)	3472 ± 471	3433 ± 306	p > 0·7
Mean R–R interval (ms)	427 ± 16	454 ± 29	p < 0·001
SD of R–R intervals (ms)	10·3 ± 2·7	13·1 ± 3·0	p < 0·001
SD of R–R interval differences (ms)	2·7 ± 1·1	6·2 ± 1·9	p < 0·001

* Student's t test (corrected for homogeneity).
The data were derived from periods of baseline heart rate only.
No distinction was drawn between active and resting patterns.
The observational epoch was 256 heart intervals.
The number of epochs per recording (mean ± SD) was 10 ± 5.
The statistical analyses were performed on the average values of the measurements for each recording (and hence each patient).

Coefficient of variation of the heart intervals

To allow comparison with similar information presented by Curran and MacGregor (1970), the coefficient of variation was measured over sequences of 1024 intervals in all the recordings between 28 and 41 weeks gestation. The data were not divided into categories for this analysis. Only 8 per cent of the results fell in the range regarded as normal by Curran and MacGregor (1970), that is at or below a value of 2·5 per cent (Fig. 6).

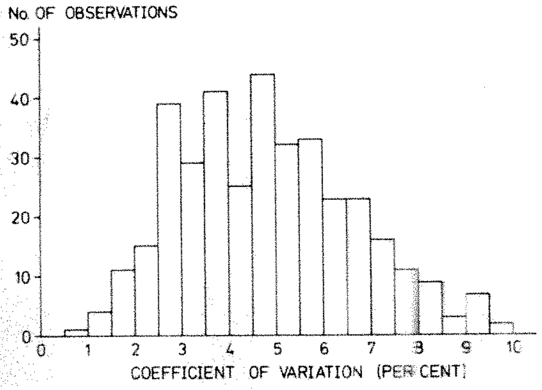


FIG. 6

Coefficient of variation of R–R intervals measured over epochs of 1024 successive intervals. The data were obtained from 61 recordings between 28 and 41 weeks gestation and have been analysed without division into categories.

Characteristics of the baseline heart rate during periods of fetal rest and activity

Thirty-two of the recordings made between 34 and 41 weeks gestation (62 per cent) showed changes in the FHR associated with periods of fetal rest and activity. These were observed in the cardiotachograms as reductions in variation of the baseline heart rate and a relative absence of accelerations during periods of rest. The values for the standard deviation of the intervals and the standard deviation of the interval differences during the periods of baseline FHR in these two states are shown in Figure 7. A statistical comparison of the measurements (Table II)

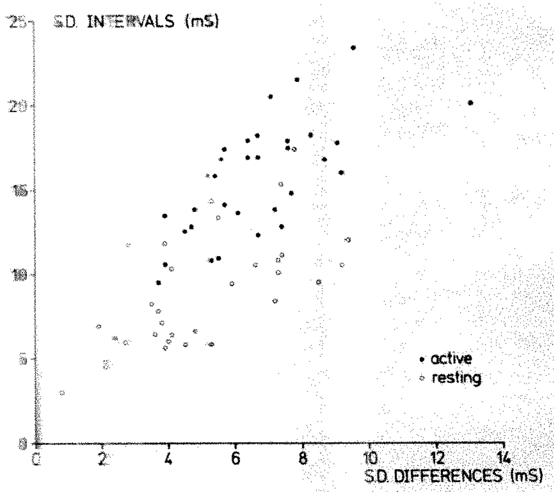


FIG. 7

The effect of fetal rest and activity on the standard deviation of the R–R intervals and the standard deviation of the R–R interval differences during baseline heart rate. The data are taken from the 32 recordings which showed changes in heart rate pattern associated with fetal rest and activity. Observational epoch: 256 successive intervals. Each data point represents the mean values of the measurements for the indicated state in a recording.

TABLE II

Comparison of the baseline heart rate during fetal rest with that during fetal activity

	Active Mean ± SD	Resting Mean ± SD	Difference Mean ± SD	Significance*
Mean R–R interval (ms)	445 ± 24	465 ± 29	20 ± 20	$p < 0{\cdot}001$
SD of R–R intervals (ms)	15·7 ± 3·3	8·9 ± 3·4	6·8 ± 2·5	$p < 0{\cdot}001$
SD of R–R interval differences (ms)	6·7 ± 2·0	5·0 ± 2·3	1·7 ± 2·4	$p < 0{\cdot}001$

* Paired t test (32 comparisons).
The observational epoch was 256 heart intervals.
The numbers of resting and active epochs per recording (mean ± SD) were 7 ± 3 and 5 ± 2 respectively.
The statistical analyses were performed on the average values of the measurements for each state in each recording.

showed that, in the resting state, the mean interval was significantly greater ($p < 0{\cdot}001$) and the standard deviations of both the intervals and the interval differences were significantly less than in the active state ($p < 0{\cdot}001$).

DISCUSSION

The signal-to-noise ratio of the human fetal ECG on the maternal abdomen limits the accuracy of the R–R interval measurements to approximately 1 ms (see 'Measurement Precision'); however, the corrections which have to be made to the measurements of R–R interval variation to allow for this, are generally small. When the R–R interval difference measurements were corrected for noise (Fig. 3), 16 per cent of them were found to lie below 1 ms, a smaller proportion than has been found in the lamb fetus where 46 per cent of the interval differences are of less than 1 ms (Dalton *et al*, 1977). More recent observations which we have made in other patients, also in late pregnancy and with similar average FHR (129 to 145 beats/minute), have shown a range for the proportion of interval differences below 1 ms of from 12 to 34 per cent, with an overall mean of 20 per cent. Thus, in this context, a distinction between the lamb and the human fetus appears to exist. However, in several other respects, this study has demonstrated similarities between the fetal heart rate of the lamb (Dalton *et al*, 1977) and that of the human.

Multiple rhythms in the human FHR were observed in the cardiotachograms described previously (Wheeler and Murrills, 1978) and have given rise to the increase in standard deviation of the heart intervals noted with increased observation time (Fig. 4). This finding emphasizes the importance of stating the period over which the measurements are made. In this study the use of a relatively short 256-interval epoch (approximately two minutes in the human fetus) enabled the baseline heart rate to be separated from accelerations and decelerations.

The decrease in heart rate as gestation advances is well recognised both in the human (Ibarra-Polo *et al*, 1972) and in the lamb (Boddy *et al*, 1974) and, since atropine abolishes this trend, the vagus nerve probably plays an important role in the process (Schifferli and Caldeyro-Barcia, 1973). This study has also shown, for the first time in the human, that FHR variation increases significantly with gestation (Table I); Dalton *et al* (1977) have reported similar observations in the lamb. The dependence of FHR variation upon an intact efferent nervous system is demonstrated by the striking reduction in variation which follows pharmacological denervation of the heart in the fetal lamb (Dalton *et al*, 1977) and in the human fetus (Méndez-Bauer *et al*, 1963; Kretowicz, 1968).

FHR variation also reflects fetal behaviour and has been shown, for example, to increase with fetal breathing in both the lamb (Dalton *et al*, 1977) and the human (Wheeler *et al*, 1978*a*). The transient increases in heart rate which accompany gross forms of fetal movement enable a cycle of rest and activity to be observed in the human fetus after 34 weeks of gestation (Wheeler and Guerard, 1974; Wheeler and Murrills, 1978). The periodicity of this cycle, which is also accompanied by significant changes in baseline heart rate variation (Table II), is similar to that of the REM/non-REM cycle in the human newborn (Sterman and Hoppenbrouwers, 1971), suggesting that the control of these phenomena is closely linked within the central nervous system (CNS). The influence of the fetal CNS on heart rate variation is also evident from the reduction in variation caused by narcotic drugs such as diazepam (Yeh *et al*, 1974), and it is likely that the 'silent' pattern of FHR, which can precede fetal death, also reflects a depression of CNS activity.

The values for the coefficient of variation in this study contrast with those found by Curran and MacGregor (1970). Most of our measurements (92 per cent) were above $2{\cdot}5$ and therefore in the range which Curran and MacGregor (1970) believed to indicate fetal compromise. Whilst it is true that the patients studied by Curran and MacGregor (1970) were drawn from a high-risk obstetric population resting in hospital, 75 per cent of the babies involved were born in good condition. Our own observations on in-patients do not suggest that the healthy fetus in this situation has a heart rate pattern which differs from that found in an out-patient group. Therefore, we cannot offer a satisfactory explanation for the difference between our much higher values for the coefficient of variation and those reported by Curran and MacGregor (1970).

This apart, Curran and MacGregor (1970) found an apparently surprising relationship between the coefficient of variation and fetal outcome as judged by Apgar score, high values of the coefficient being associated with low scores. This result appears to be at variance with the theory of Hammacher *et al* (1968) that a lack of heart rate variation, as seen in the silent pattern, is associated with fetal jeopardy. Several papers since that of Hammacher *et al* (1968) have confirmed a close relationship between the silent pattern of FHR and fetal death during pregnancy (Kubli *et al*, 1972; Tushuizen *et al*, 1974; Simmons, 1974; Emmen *et al*, 1975). Because the measurements of Curran and MacGregor (1970) were made over arbitrary ten-minute periods, without visualization of the heart rate patterns before the computer analyses, it is possible that major departures from the baseline such as decelerations were included. These could have caused the relatively high values for the coefficient of variation associated with poor outcome. (Hammacher and others account separately for decelerations in their assessments of the FHR). We now also know that a ten minute sample of FHR is too short to be representative (Wheeler and Murrills, 1978) and this may have been a factor contributing to the rather poor correlation (r = −0·5) between Apgar score and coefficient of variation in the results of Curran and MacGregor (1970).

In experiments on fetal lambs, both Dalton *et al* (1977) and Stånge *et al* (1977) have observed that an increase in heart rate variation accompanies hypoxaemia. Although this finding tends to support the view of Curran and MacGregor (1970), it is apparently in disagreement with the human evidence cited above; however, the human and animal data have been obtained under different conditions. In the lamb experiments, healthy animals have been subjected to acute hypoxaemia, whereas the human data have been collected from sick fetuses exposed to an unfavourable intrauterine environment for a prolonged period of time. Interestingly, Martin (1977) has observed that a healthy fetal monkey subjected to acute hypoxaemia shows an increase in heart rate variation, but that a reduction in variation occurs if the hypoxaemia is prolonged; this phenomenon was also observed by Dalton *et al* (1977) in the one of two fetal lambs subjected to relatively prolonged hypoxaemia which also became acidaemic.

In this study, long and short term variation in baseline heart rate have been measured respectively by the standard deviation of the intervals and the standard deviation of the interval differences. These two parameters may be compared with the expressions of Yeh *et al* (1973). Their measure of long term variation, the 'interval index', is simply the coefficient of variation of the heart intervals, whilst for short

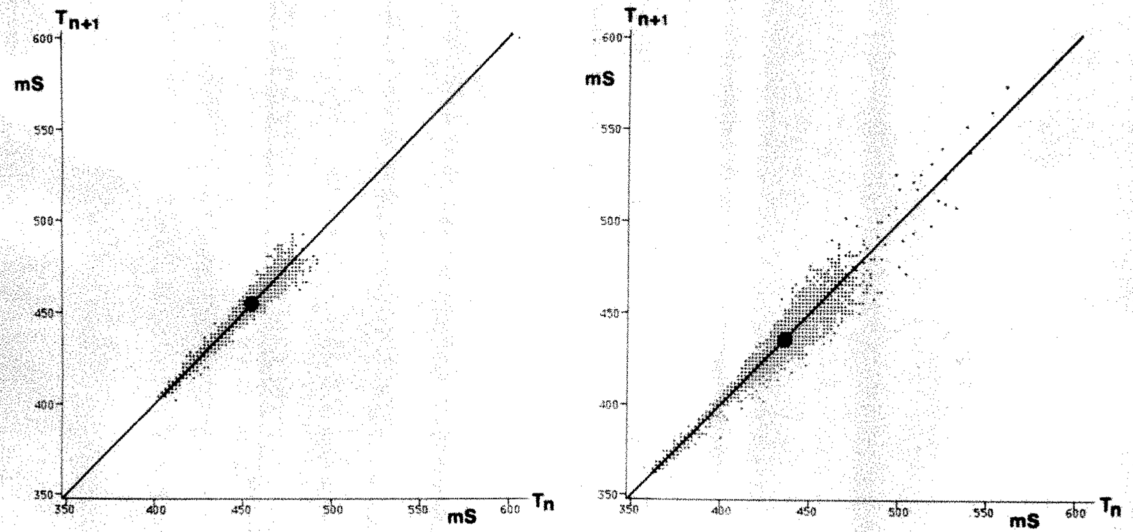
TABLE II

Some expressions used to describe heart rate variation. (T_1, T_2, T_3 . . . *etc. indicate successive R–R intervals*)

Long Term Variation	*Short Term Variation*
1. Standard deviation of R–R intervals: Dalton *et al*, 1977 (various stated epochs) This study (various stated epochs)	1. Standard deviation of R–R interval differences: i.e. SD of $T_1 - T_2, T_2 - T_3, \ldots$ etc.: This study
2. Coefficient of variation of R–R intervals: i.e. $\frac{\text{SD}}{\text{mean}}$ Curran and MacGregor, 1970 (epoch: about ten minutes) Yeh *et al*, 1973 (epoch: 30 seconds)	2. Mean absolute 'beat-by-beat' difference: i.e. mean of $\frac{\lvert T_1 - T_2 \rvert}{2}, \frac{\lvert T_2 - T_3 \rvert}{2}, \ldots$ etc.: Dalton *et al*, 1977
	3. 'Differential Index': i.e. $1000 \times \text{SD of } \frac{T_1 - T_2}{T_1 + T_2}, \frac{T_2 - T_3}{T_2 + T_3}, \ldots$ etc.: Yeh *et al*, 1973

term variation they have devised a 'differential index' which relates each interval difference to the mean of the intervals producing it before the standard deviation is calculated (Table III). The inherently close correlation which exists between these indices and our own, within the normal limits of FHR, is not maintained over the full fetal range because both expressions of Yeh *et al* (1973) involve division by the mean heart interval. On the other hand the close correlation ($r = 0 \cdot 97$) that we also observed between the mean absolute beat-by-beat difference of Dalton *et al* (1977) (Table III) and the standard deviation of interval differences might be expected to hold over a wide range of mean heart rate.

The relationship between heart rate variation and mean heart rate has in fact provoked controversy (de Haan *v* Dalton, 1977). This would seem to have arisen both from the inconsistent nature of the relationship and from differences in definition. This study has shown that both the mean heart rate and its variation increase with a change in fetal state from rest to activity (Table II) and Dalton *et al* (1977) have reported the same association between mean rate and variation in the context of diurnal variation in the fetal lamb. However, de Haan (1977) has apparently expressed the opposite view by stating that, both in the human and in the fetal lamb, 'the higher the basal heart rate, the lower is the short-term variability' (see also de Haan *et al*, 1972). Figure 8 shows human fetal heart intervals plotted according to the system of de Haan *et al* (1971) with the data for a period of rest separated from those for a period of activity. Because successive intervals are plotted against each other, short term variation (from beat to beat) is represented by the scatter of points to each side of the line of equality, whilst long term variation produces scatter along the line. The greater variation, both long and short term, is associated with the higher mean heart rate found in the active state. However, *within* each state the longer intervals are associated with the greater beat-to-beat variation, this giving rise to the wedge-shaped plots. A



RESTING

Overall mean [●]	**454 mS**
S D Intervals	**16 mS**
S D Differences	**4 mS**

ACTIVE

Overall mean [●]	**435 mS**
S D Intervals	**25 mS**
S D Differences	**7 mS**

FIG. 8

Fetal heart rate characteristics during a 20 minute period of rest and a 20 minute period of activity in a fetus of 41 weeks gestation. The data are plotted according to the two dimensional system of de Haan *et al* (1971); T_n = duration of nth heart interval. The overall values for the mean heart interval and the standard deviations of intervals and interval differences are given for each state.

fundamental difference between these two situations is in the period of time over which the 'mean' heart rate is calculated; in the former this is several minutes but in the latter it comprises no more than two successive heart intervals. In the context of this 'instantaneous' mean, the statement of de Haan (1977) is obviously correct and is illustrated by the reduction in variation commonly occurring at the peak of a heart rate acceleration. There are also situations in which a negative correlation exists between heart rate variation and mean heart rate measured over a relatively long period. For example, heart rate variation increases whilst mean rate decreases with gestation (Dalton *et al*, 1977; Wheeler and Murrills, 1978), and the initial response to hypoxia in the fetal lamb is an increase in variation accompanied by a decrease in mean rate (Dalton *et al*, 1977). An example of an inconsistent relation between heart rate variation and mean heart rate occurs with fetal breathing. Dalton *et al* (1977) have observed an almost universal increase in variation with the onset of breathing in the fetal lamb but have found the direction of the change in mean rate to be unpredictable; our own studies in the human agree with this result (Wheeler *et al*, 1978*a*). Confusion in this area can only be avoided by precise definition of measurement parameters and observational context and, in view of the apparently complex relationship between the mean fetal heart rate and its variation, expressions which combine the two need to be formulated with care.

The possible advantages of computer analysis of the FHR should be examined in the context of the practical needs of the obstetrician. Help is most needed at present in the objective description of heart rate variation in antenatal cardiotocograms. The visual classification proposed by Hammacher *et al* (1968) lacks objectivity and the more recent proposal from Hammacher *et al* (1974) of a division of heart rate variation into 12 categories, whilst attempting to distinguish between long and short term variation, is difficult to implement because the assessment still has to be made by eye. Automatic measurements are hampered by the presence of artifactual variation in records obtained by ultrasound or phonocardiography but, with careful exclusion of these, a computer could at least assess the long term variation. Measurements of the relatively small beat-to-beat variation at present continue to depend upon fetal ECG signals, but the poor signal-to-noise ratio often encountered when these are recorded from the maternal abdomen precludes universal application of the technique.

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HAEMODYNAMICS OF MILD HYPERTENSION IN PREGNANCY

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Summary

One hundred and sixty-three haemodynamic studies were done serially in 23 normotensive and 25 mildly hypertensive women during pregnancy. With all subjects' resting in the left lateral position, heart rate, cardiac output, stroke volume, left ventricular work index, total peripheral vascular resistance and haematocrit were all significantly higher while plasma volume per unit body weight was significantly lower in the hypertensive than in the normotensive pregnant women. It is postulated that this hyperkinetic circulatory state is due to hyperactivity of the sympathetic adrenergic component of the autonomic nervous system in hypertensive pregnancy.

Early essential hypertension in non-pregnant subjects is characterized by alterations in central haemodynamics, notably an increase in cardiac output (Julius and Schork, 1971). Disorders in the control of cardiac output are thought by some workers to initiate the development of essential hypertension (Frohlich, 1973).

While an increase in cardiac output is an essential component of physiological adaptation to pregnancy, the resulting hyperkinetic circulation resembles the haemodynamic state in non-pregnant subjects with early borderline essential hypertension.

Cardiac output in hypertensive pregnancy has been reported variously as being increased (Hamilton, 1949), showing no significant change (Assali *et al*, 1964), or being reduced (Smith, 1970). Hence, an attempt was made to elucidate the haemodynamic characteristics of gestational hypertension that occurs in young women and is, therefore, often in an early phase of development. Thereby, it was hoped to gain some insight into the genesis of hypertension with a view to prevention or the use of rational antihypertensive therapy.

Materials and Methods

Twenty-three normotensive and 25 mildly hypertensive pregnant women attending the antenatal clinic volunteered for this study. Their ages ranged from 17 to 40 years and the study intervals extended from 6 to 41 weeks gestation. Of the 23 normotensive controls, 16 were primigravidae and 7 multigravidae: the corresponding numbers in the hypertensive group were 11 and 14 respectively.

None of the control subjects had any medical or obstetric complications of pregnancy nor did they have a history of previous disease. Amongst the hypertensives, none of the 11 primigravidae had any relevant medical history or apparent cause for hypertension. Nine of the 14 hypertensive multigravidae had a history of benign essential hypertension in previous pregnancies, while the other five lacked any evidence of previous hypertension.

The criterion for inclusion in the hypertensive group was a mean arterial blood pressure of 100 mm Hg or more.

All subjects received a thorough physical examination including urinalysis at the first and subsequent antenatal visits. Blood levels of urea, electrolytes, creatinine, uric acid and glucose, and urinary levels of protein, electrolytes and creatinine, were measured in all subjects on two or more occasions during pregnancy. A single chest radiograph and serial electrocardiograms were also obtained. All of these investigations were within normal limits. Thus it was concluded that all of the hypertensive subjects in this study were most likely to have had early mild benign essential hypertension.

Haemodynamic studies were performed at intervals of four weeks for an average period of six months. Each subject had at least three studies performed during pregnancy. Subjects were asked to refrain from smoking and undue exertion and to avoid eating for at least two hours before each study, which was conducted in a quiet room maintained at a temperature of 22°C. After measuring body height and weight, duplicate readings of brachial arterial blood pressure were made by indirect sphygmomanometry in the sitting, standing and supine positions. The subject was then turned into the left lateral position while blood pressure, heart rate, plasma volume and cardiac output measurements were made.

Cardiac output was determined by the photoelectric earpiece dye-dilution method of Gabe and Shillingford (1961) as described by Walters *et al* (1966). Evans blue (T1824) was the dye chosen because of its suitable characteristics for both cardiac output and plasma volume measurements (Walters *et al*, 1966). Recovery of the dye from plasma was achieved by the extraction method of Tornberg (1958).

The venous haematocrit was measured by the microhaematocrit method using the Hawksley equipment (Hawksley and Sons Ltd, London, England). Mean arterial blood pressure, left ventricular work index, total peripheral resistance index, mean systolic ejection rate, cardiac index, stroke index, plasma volume index and total blood volume were calculated secondarily from the primary measurements described above using standard formulae.

Twenty of the hypertensive pregnant women had mild hypertension only throughout pregnancy while the other five developed generalized oedema and proteinuria in the late second or third trimesters.

Most of the subjects who developed a rise in blood pressure above 140/90 mm Hg were treated with bed rest and mild nocturnal sedation (barbiturate) at home or in hospital. Only five hypertensive subjects required antihypertensive drugs (α-methyldopa or clonidine) during later stages of pregnancy: data obtained from these patients after commencement of therapy were excluded from the study.

Statistical analysis of the data, using a computer, included the determination of the mean and standard deviation for each variable and an unpaired t-test of differences between each variable in the two experimental groups. Factorial analysis was used to determine the significance of factors affecting each variable and differences between each variable in the two groups were determined by covariance analysis. Linear regression lines for the various haemodynamic variables were constructed and regression equations compared using the t-test for slope and location.

RESULTS

One hundred and sixty-three haemodynamic studies were carried out in 48 subjects during pregnancy. The number of studies in each gestational period is listed in Table I.

Mean age, gestational period, body weight and surface area (Table I)

The age (mean ± SD; 23·4 ± 5·8 years) of the 23 normotensive pregnant subjects was significantly lower than that (26·9 ± 6·7 years) of the hypertensives ($p < 0{\cdot}001$).

The overall mean length of pregnancy (mean ± SD) during which patients were studied was not significantly different between the two groups, being 28·5 ± 7·5 weeks in the normotensives and 29·1 ± 6·8 weeks in the hypertensives.

The latter were heavier, with a body weight (mean ± SD) during pregnancy of 80·07 ± 17·6 kg and larger, with a body surface area during pregnancy of $1{\cdot}81 \pm 0{\cdot}21$ m^2, compared with the normotensive controls in whom the corres-

TABLE I

Age, gestational period, body weight, body surface area and number of patients in each gestational subgroup of normotensive and hypertensive women

Patient category	Age (years) Mean ± 1SD	Gestational age at time of study (weeks) Mean ± 1SD	Body weight (kg) Mean ± 1SD	Body surface area (m²) Mean ± 1SD	No. of observations	No. of patients in each gestational subgroup (weeks)						
						0–16	17–20	21–24	25–28	29–32	33–36	37–40
Normotensive pregnancy	23·4 ± 5·8	28·5 ± 7·5	68·56 ±11·88	1·712 ±0·130	86	4	12	13	12	16	14	15
Hypertensive pregnancy	26·9 ± 6·7	29·1 ± 6·8	80·07 ±17·52	1·81 ±0·21	77	3	7	12	14	13	14	14
t value	** 3·51	0·50 (N.S.)	** 4·93	** 3·62	—	—	—	—	—	—	—	—

N.S. = Not statistically significant
** = p < 0·001

TABLE II

Differences in brachial arterial blood pressure between normotensive and hypertensive pregnant women

Variable	Normotensive pregnancy (Mean ± 1SD)	Hypertensive pregnancy (Mean ± 1SD)	t value	Covariance analysis			
					Adjusted F value with covariables		
				Unadjusted F value	Age (years)	Gestational age (weeks)	Body weight (kg)
Systolic arterial pressure (mm Hg)	115·2 ± 8·7	137·2 ± 15·3	**11·42	130·5	**122·7	**131·9	**122·8
Diastolic arterial pressure (mm Hg)	71·3 ± 5·8	90·9 ± 6·7	**13·20	175·0	**169·3	**179·5	**160·3
Arterial pressure (mm Hg)	85·9 ± 7·5	106·3 ± 11·5	**13·56	183·8	**175·7	**184·5	**168·4

** p < 0·001

ponding figures were 68·56 ± 11·88 kg and 1·71 ± 0·13 m², respectively ($p < 0·001$).

Brachial arterial blood pressure

Systolic, diastolic and mean arterial blood pressures, measured with patients in the left lateral position, were all significantly higher in the hypertensive subjects (Table II).

Heart rate

Heart rate at rest increased progressively throughout pregnancy in both groups of pregnant subjects. Despite a wider variation, the overall resting heart rate (mean ± SD) in the hypertensive subjects was significantly higher than that of the normotensives, being 91·2 ± 14·0 beats per minute in the former and 79·9 ± 10·4 beats per minute in the latter ($p < 0·001$). The difference was more marked in the second half of pregnancy and remained significant after adjusting for variation in age, body weight and gestational period (Table III).

Cardiac output

The overall cardiac output (mean ± SD) for all gestational periods was greater (8·77 ± 2·09

TABLE III

Differences in heart rate, cardiac output and stroke volume between normotensive and hypertensive pregnant women

Variable	Normotensive pregnancy Mean ± 1SD	Hypertensive pregnancy Mean ± 1SD	t value	Covariance analysis		
				Unadjusted F value	Covariable	Adjusted F value
Heart rate (beats/minute)	79·9 ±10·4	91·2 ±14·0	**5·85	9·69	Age	** 6·86
					Gestational age	** 8·67
					Body weight	** 7·00
Cardiac output (l/minute)	7·80 ±1·38	8·77 ±2·09	**3·51	12·40	Gestational age	**12·27
					Body surface area	* 4·34
					Arterial pressure	2·78 (N.S.)
					Heart rate	**13·52
					Stroke volume	2·31 (N.S.)
					Plasma volume	2·48 (N.S.)
Stroke volume (ml)	92·8 ±16·1	104·3 ±25·5	**3·49	12·20	Gestational age	**11·99
					Heart rate	**15·29
					Arterial pressure	2·78 (N.S.)
					Body weight	* 4·25

N.S. = Not statistically significant * = p < 0·05 ** = p 0·001

TABLE IV

Comparison of linear regression equations of cardiac output versus gestational period, heart rate, stroke volume, total peripheral resistance index and plasma volume and stroke volume versus heart rate and plasma volume in normotensive and hypertensive pregnant women

Patient category	Regression equation	Correlation coefficient	t value	t value of difference in slope	t value of difference in location
Normotensive	C.O. = 6·355 + 0·051 × G.P.	0·28	† 2·84	−0·027 (N.S.)	**−0·93
Hypertensive	C.O. = 6·512 + 0·078 × G.P.	0·25	* 2·25		
Normotensive	C.O. = 3·65 + 0·049 × H.R.	0·39	** 3·92	−0·003 (N.S.)	**−0·93
Hypertensive	C.O. = 4·32 + 0·052 × H.R.	0·39	** 3·65		
Normotensive	C.O. = 2·25 + 0·06 × S.V.	0·70	** 8·92	0·003 (N.S.)	−0·30 (N.S.)
Hypertensive	C.O. = 2·85 + 0·056 × S.V.	0·69	** 8·32		
Normotensive	C.O. = 13·59 − 0·0038 × T.P.R.I.	−0·70	**−9·10	−0·0003 (N.S.)	**−1·98
Hypertensive	C.O. = 15·04 − 0·0034 × T.P.R.I.	−0·71	**−8·70		
Normotensive	C.O. = 1·996 + 1·711 × P.V.	0·55	5·97	−0·51 (N.S.)	−0·31 (N.S.)
Hypertensive	C.O. = 0·524 + 2·22 × P.V.	0·77	** 10·40		
Normotensive	S.V. = 139·0 − 0·55 × H.R.	−0·38	**−3·72	0·07 (N.S.)	‡−3·89
Hypertensive	S.V. = 157·2 − 0·62 × H.R.	−0·38	**−3·56		
Normotensive	S.V. = 30·86 + 18·24 × P.V.	0·50	** 5·29	−2·90 (N.S.)	−1·81 (N.S.)
Hypertensive	S.V. = 25·84 + 21·14 × P.V.	0·60	† 6·53		

C.O. = Cardiac output, G.P. = Gestational period, H.R. = Heart rate, S.V. = Stroke volume, T.P.R.I. = Total peripheral resistance index, P.V. = Plasma volume.

N.S. = Not statistically significant, * = p < 0·05, † = p < 0·02, ‡ = p < 0·01, ** = p < 0·001 l/minute) in the hypertensive than in the normotensive group (7·80 ± 1·38 l/minute). This difference was statistically significant ($p < 0\cdot001$) and remained so to a lesser degree ($p < 0\cdot05$) after adjustment for variation in body size between the two groups (Table III). The cardiac output correlated more closely with stroke volume than with heart rate as shown by covariance analysis using each variable separately (Table III).

A significant inverse relationship existed between cardiac output and the total peripheral resistance index within each group while a positive relationship was found between cardiac output and plasma volume. However, comparison of the relationship between cardiac output and total peripheral resistance index, and cardiac output and plasma volume between the two groups revealed no significant difference (Table IV).

Stroke volume

Stroke volume changes closely followed those of cardiac output in both groups, the overall stroke volume (mean ± SD) being greater (104·3 ± 25·5 ml) in the hypertensive than in the normotensive (92·8 ± 16·1 ml) group ($p < 0\cdot001$). Although the difference in mean body weight between the two groups modified the degree of this finding, it remained significant ($p < 0\cdot05$). Mean arterial pressure was a strong determinant of the difference in stroke volume between the two groups (Table III). In addition, stroke volume varied directly with plasma volume but inversely with heart rate (Table IV).

Left ventricular work index

Since both cardiac output and mean arterial pressure were higher in the hypertensive subjects it follows that the work done by the heart must have increased. This was confirmed by values for left ventricular work index (mean ± SD) of (516·7 ± 124·7 mmHg l/minute m²) in the hypertensive and (391·7 ± 73·9 mmHg l/minute m²) in the control group ($p < 0\cdot001$). However, the statistical significance of this difference was abolished when mean arterial pressure differences were taken into account, indicating that the higher left ventricular work index in the hypertensive subjects was predominantly an effect of higher systemic arterial pressure (Table V).

Systolic ejection rate

Although the systolic ejection rate (mean ± SD) was numerically higher in the hypertensive (212·6 ± 46·9 ml/second m²) than in the normotensive (202·1 ± 32·3 ml/second m²) group, the difference was not significant ($0\cdot02 > p > 0\cdot05$). Variation in body weight could have obscured the true difference (Table VI).

TABLE V

Differences in left ventricular work index, mean systolic ejection rate and total peripheral resistance index between normotensive and hypertensive pregnant women

Variable	Normotensive pregnancy Mean ± 1SD	Hypertensive pregnancy Mean ± 1SD	t value	Covariance analysis: Unadjusted F value	Covariable	Adjusted F value
Left ventricular work index (mm Hg l/minute m²)	391·7 ± 73·9	515·7 ± 124·7	**7·87	52·0	Gestational age Arterial pressure Body weight	**62·92 < 1 (N.S.) **56·53
Mean systolic ejection rate (ml/m² second)	202·12 ± 32·18	212·61 ± 46·93	1·68	2·80	Gestational age Arterial pressure Body weight	2·63 (N.S.) 1·31 (N.S.) * 4·37
Total peripheral resistance index (dynes/second/cm⁵m²)	1540 ± 258	1828 ± 434	**5·21	**27·13	Gestational age Body weight Arterial pressure Haematocrit	**27·87 **23·55 < 1 (N.S.) **17·76

N.S. = Not statistically significant * = p <0·05 ** = p <0·001

TABLE VI

Differences in venous haematocrit, plasma volume, plasma volume index and total blood volume between normotensive and hypertensive pregnant women

Variable	Normotensive pregnancy Mean ± 1SD	Hypertensive pregnancy Mean ± 1SD	t value	Covariable analysis		
				Unadjusted F value	Covariable	Adjusted F value
Haematocrit (per cent)	34·16 ± 2·62	36·19 ± 2·72	**4·84	23·40	Gestational age	**23·33
					Body weight	**17·90
Plasma volume (l)	3·394 ± 0·440	3·711 ± 0·726	**3·42	11·67	Gestational age	**12·90
					Body weight	< 1 (N.S.)
					Body surface area	2·38 (N.S.)
Plasma volume index (l/kg)	50·14 ± 6·36	47·47 ± 9·06	**2·19	4·80	Gestational age	* 5·65
					Arterial pressure	< 1 (N.S.)
Total blood volume (l)	4·935 ± 0·691	5·529 ± 1·028	**4·37	19·10	Gestational age	**21·90
					Body weight	3·41 (N.S.)

N.S. = Not statistically significant * = $p < 0{\cdot}05$ ** = $p < 0{\cdot}001$

Total peripheral resistance index

The total peripheral resistance index (mean ± SD) was significantly higher in the hypertensive subjects (1828 ± 434 dynes seconds/cm^5 m^2) throughout pregnancy than in the normotensive controls (1540 ± 258 dynes seconds/cm^5 m^2). The significance of this difference ($p < 0{\cdot}001$) remained unchanged after adjustment for variations in gestational period and body weight (Table V).

Venous haematocrit

The overall venous haematocrit level (mean ± SD) was significantly higher in the hypertensive (36·19 ± 2·72 per cent) compared with the normotensive (34·16 ± 2·62 per cent) group ($p < 0{\cdot}001$). This difference was independent of gestational period and body weight variations between the two groups (Table VI).

Plasma volume

Plasma volume increased progressively throughout pregnancy in both hypertensive and normotensive subjects. Furthermore, the overall plasma volume (mean ± SD) was greater in the hypertensive (3·711 ± 0·726 l) than in the normotensive (3·394 ± 0·440 l) group. However, since body weight correlated positively with plasma volume in both groups, the difference in plasma volume became statistically insignificant after adjusting for body weight variation (Table VI). Indeed, mean plasma volume per unit body weight was slightly but significantly smaller in the hypertensive group (Table VI).

Total blood volume

Total blood volume, calculated from plasma volume and the total body haematocrit, showed changes that ran almost parallel with those of plasma volume in both groups. Again, body weight influenced the higher total blood volume found in the hypertensive group. In fact, the difference in mean total blood volume between the two groups was not significant after covariance analysis using body weight as a covariable (Table VI).

DISCUSSION

This study has demonstrated significant differences between the haemodynamic characteristics of normotensive and mildly hypertensive pregnant women. The latter showed hyperkinetic cardiac function, manifested by a faster heart rate and higher cardiac output, exceeding that observed in normal pregnancy. The increase in cardiac output was due more to enhancement of stroke volume than to an increase in heart rate. In addition, the hypertensive pregnant women had a higher total peripheral resistance and a smaller plasma volume per unit body weight compared with the normotensive controls. These findings indicate that blood pressure regulation is altered at both central and peripheral sites in hypertensive pregnant women.

In hypertension associated with a high cardiac output, a preferential central distribution of circulating blood volume rather than its total amount is thought to be the effective initiating factor (Ellis and Julius, 1973). Furthermore, in established essential hypertension there is a progressive diminution in plasma volume as arterial pressure rises (Tarazi *et al*, 1970). In the hypertensive pregnant women in this study, although plasma volume per unit body weight was smaller in the control group, central distribution of total circulating blood volume could have been larger. Minor degrees of peripheral vasoconstriction, especially in the venous circulation, could have sufficed to shift circulating blood to the heart from the periphery without at first affecting total plasma volume.

A predominant neurogenic regulation of blood pressure has been reported in hypertensive pregnancy (Castrén *et al*, 1973). Tachycardia, which is a characteristic feature, has also been reported in non-pregnant subjects with fixed essential hypertension and normal cardiac output (Korner *et al*, 1973) and in chronic hypertensives with a hyperkinetic heart (Ibrahim *et al*, 1975). In the latter group, increased cardiac output, heart rate and myocardial contractility are thought to be associated with autonomic nervous dysfunction, which results in combined inhibition of the parasympathetic and stimulation of the sympathetic nervous system (Korner *et al*, 1973).

The markedly raised total peripheral resistance in hypertensive pregnant women observed in this study confirms the findings of previous workers (Hamilton, 1949; Assali *et al*, 1964). Both autonomic and humoral mechanisms contribute to this stage of peripheral vasoconstriction.

It is noteworthy that most of the abnormal haemodynamic findings observed in hypertensive pregnancy in this study can be attributed to enhanced activity of the sympathetic nervous system over and above that seen in normal pregnancy. Since oestrogen, which is produced in large amounts during pregnancy has a vasodilator action, it has been postulated that sympathetic tone increases to counteract the effects of the excess oestrogen circulating (Hytten and Leitch, 1971). Similar haemodynamic findings of lesser degree occur when normotensive women take oral contraceptives containing more than 50μg oestrogen (Walters and Lim, 1970). Hypertension may result when this pregnancy or oestrogen adaptation response of the autonomic nervous system is excessive.

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THE POSSIBLE ROLE OF PROSTAGLANDINS IN THE HYPERFUNCTION OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN THE NEWBORN

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Summary

Plasma renin activity and plasma aldosterone concentration were measured by radioimmunoassay in eight newborn infants born vaginally to mothers on indomethacin treatment. Measurements were made in venous cord blood at birth and on the sixth day of life. The results were compared to those obtained in eight healthy control newborn infants of similar gestational age and birth weight. Maternal treatment with indomethacin was associated with a decrease of plasma renin activity (mean ± SD) from 16·71 ± 2·76 ng/ml/hour in the control group to 9·60 ± 3·84 ng/ml/hour in the treated group. Plasma aldosterone concentration was lower in the cord blood of the treated group and a similar trend was demonstrated in the sixth day samples. It is suggested that the increased endogenous prostaglandin production during labour may be one of the factors responsible for the development of hyperactivity of renin-angiotensin-aldosterone system in the newborn.

IN view of several reports on the physiological hyponatremia of the low birth weight newborn infant (Day *et al*, 1976; Honour *et al*, 1977; Sulyok, 1971) it was of particular interest to study the function of the renin-angiotensin-aldosterone system (RAAS) during the neonatal period.

It has recently become evident that the activity of RAAS is greatly elevated in the newborn period (Beitins *et al*, 1972; Dillon *et al*, 1976; Godard *et al*, 1976; Hayduc *et al*, 1972; Katz *et al*, 1974; Kotchen *et al*, 1972; Lumbers and Reid, 1977; Mott, 1975; Pipkin and Smales, 1975; Pipkin and Symonds, 1977; Raux-Eurin *et al*, 1977; Siegel *et al*, 1974). The aetiology and clinical significance, however, remained to be determined. On basis of the relation between prostaglandins and renin release (Carlson *et al*, 1969; Frölich *et al*, 1975; Gill *et al*, 1976; Larsson *et al*, 1974; Werning *et al*, 1971), as well as between prostaglandins and aldosterone secretion (Saruta and Kaplan, 1972) we made an attempt to obtain information as to whether or not the increased endogenous prostaglandin production during labour might play a role in producing hyperactivity of RAAS in the newborn infant.

In the present study we compared the plasma renin activity (PRA) and plasma aldosterone (PA) concentration of newborn infants whose mothers were treated with indomethacin before delivery with a control group without such maternal treatment.

SUBJECTS AND METHODS

Eight newborn infants whose mothers were treated with indomethacin were selected for the study. Indomethacin treatment was applied according to the protocol of Zuckerman *et al* (1974) and Reiss *et al* (1976) in an attempt to prevent premature delivery or to control uterine activity at term. Indomethacin was given in a dose of 100 mg/day for a period of 1 to 3 days, the last dose being taken 8 to 24 hours before delivery.

The mean age, prepregnant weight and the weight gain of the pregnant women were 23·2 years, 54·5 kg and 11·5 kg with the ranges of 19 to 26 years, 49 to 63 kg and 7 to 15 kg respectively.

The mothers were on normal diets without diuretic therapy. None had a history of renal disease or previous toxaemia of pregnancy, and all had an uncomplicated vaginal delivery.

The mean birth weight and gestational age of the infants was 3348 g and 38·5 weeks with the ranges of 2750 to 4130 g and 35 to 41 weeks, respectively. Gestational age was assessed primarily from the mother's menstrual history but was confirmed by physical examination of the infant. The one-minute Apgar score was more than 7 and the perinatal course remained uneventful in 5 of the 8 infants. The other three infants had a one-minute Apgar score of less than 7 and symptoms consistent with the persistence of fetal circulation syndrome (Csaba *et al*, 1977 and 1978). In two babies there was moderate oedema on the first three days of life, and one baby had a serum bilirubin level of 16 mg/100 ml (274 μmol/l) on the fourth day. No haematological abnormalities were observed in the platelet count or blood coagulation.

Eight healthy newborn infants whose mothers had normal pregnancies and vaginal deliveries without indomethacin treatment served as controls. The birth weight (mean 3152 g; range 2400 to 3600 g) and gestational age (mean 38·3 weeks; range 34 to 41 weeks) were essentially the same as their treated matches. The purpose of the study was explained to each mother and informed consent was obtained for blood sampling.

Blood samples were taken from the umbilical vein before clamping of the umbilical cord by needle puncture and by venepuncture at 1000 hours on the sixth day of life. The sixth day sample was taken three hours after the last feed and the baby was kept supine for one to three hours. After the samples were drawn into syringes they were immediately injected into tubes in ice water containing EDTA (for PRA estimation) or heparin (for PA measurement). Plasma was separated in a refrigerated centrifuge and stored at −20°C.

PRA was measured by the radioimmunoassay of generated angiotensin I (Haber *et al*, 1969) the PA concentration was determined by radioimmunoassay (Vetter, 1973) using SORIN-CEA-IRE (Italy) RIA kits. The normal values in supine adults and the sensitivity of the methods in our laboratory are 0·9±0·75 ng/ml/hour (mean±SD) and 0·2 ng/ml/hour for PRA and 112±25 pg/ml and 4 pg/ml for PA, respectively.

Plasma sodium and potassium concentrations were measured by flame photometry.

As most of the newborn infants were female, exact urine collection could not be done, therefore urinary electrolyte excretion was not measured. However, each infant studied was on a cow's milk formula providing sodium and potassium intakes of 1·2 and 2·2 m mol/kg/day in the treated and 1·3 and 2·4 m mol/kg/day in the control group, respectively. Statistical evaluation was done by using Student's t test.

RESULTS

Figure 1 shows that the PRA of healthy newborn infants born to untreated mothers fell markedly from a mean of 16·7 ng/ml/hour in the cord blood at birth to a mean of 8·2 ng/ml/

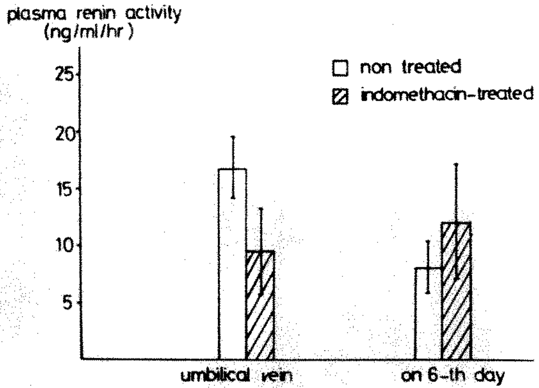

FIG. 1

Effect of maternal indomethacin treatment on plasma renin activity (PRA) in venous cord blood and on the sixth day of neonatal life. Vertical bars represent the standard errors of the means.

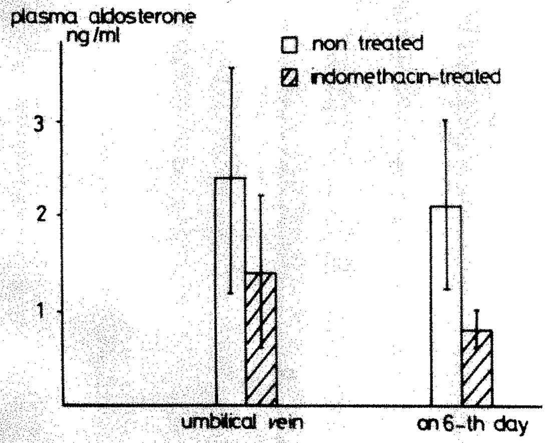

FIG. 2

Effect of maternal indomethacin treatment on plasma aldosterone (PA) in venous cord blood and on the sixth day of neonatal life.

hour on the sixth day of life. Maternal treatment with indomethacin was associated with an apparent decrease of PRA in the venous cord blood. However, such difference could not be observed on the sixth postnatal day. The changes in PA concentration are shown in Figure 2. It can be seen that the mean PA of 2·4 ng/ml in venous cord blood of infants born to untreated mothers remained practically unchanged during the study period of six days.

As a result of maternal indomethacin treatment there was a decrease in PA in both the venous cord blood and the sixth day samples. This decrease, however, just fell short of statistical significance.

There was no difference in plasma sodium and potassium concentrations between the treated and control group either in the cord blood or in the sixth day samples (Table I).

DISCUSSION

On the basis of the widespread use and apparent clinical acceptance of maternal treatment with prostaglandin-antagonists for preventing premature delivery or controlling uterine activity at term (Győry *et al*, 1974; Manchester *et al*, 1976; Reiss *et al*, 1976; Zuckerman *et al*, 1974), it was thought that such treatment might be introduced into our clinical practice and provide information about the role of prostaglandins in the development of hyperactivity of the renin-angiotensin-aldostérone system (RAAS) during the neonatal period.

The high incidence of persistence of fetal circulation in infants born to mothers on indomethacin treatment drew our attention to its potential hazard to the newborn (Csaba *et al*,

TABLE I

Effect of maternal treatment with indomethacin on the plasma sodium and potassium concentration of cord vein blood and venous blood obtained from the infant when six days old

	Indomethacin—treated infants		Control infants	
	Venous cord blood	Sixth day	Venous cord blood	Sixth day
Plasma sodium (mmol/l) Mean ± SE	140·1 ± 1·5	139·6 ± 0·7	142·1 ± 2·3	140·5 ± 1·0
Plasma potassium (mmol/l) Mean ± SE	4·9 ± 0·3	4·6 ± 0·2	5·3 ± 0·5	4·3 ± 0·3

1977 and 1978; Manchester *et al*, 1976) and this study was therefore discontinued.

Although our findings only demonstrate a trend and the numbers are not sufficiently large to allow us to make a definitive conclusion, the results suggest that the increased endogenous prostaglandin production during labour may be intimately involved in the increased activity of RAAS in the newborn infant.

Prostaglandins may stimulate the release of renin and the biogenesis of aldosterone (Carlson *et al*, 1969; Frölich *et al*, 1975; Gill *et al*, 1976; Larsson *et al*, 1974; Saruta and Kaplan, 1972; Werning *et al*, 1971).

Indomethacin, a potent inhibitor of prostaglandin synthesis, has been reported to decrease PRA (Frölich *et al*, 1975; Gill *et al*, 1976; Larsson *et al*, 1974; Romero *et al*, 1976). During labour, endogenous prostaglandin production is greatly increased (Green *et al*, 1974; Hillier *et al*, 1974; Karim and Devlin, 1967; Karim, 1968; Sharma *et al*, 1973) and labour associated with vigorous uterine activity (Batra and Bengtsson, 1976) is also associated with a higher rate of prostaglandin production than that associated with delivery by elective Caesarean section (Jonsson *et al*, 1976).

Renin activity (Lammintausta *et al*, 1977; Lumbers and Reid, 1977), angiotensin (Lammintausta *et al*, 1977; Lumbers and Reid, 1977; Pipkin and Symonds, 1977) and aldosterone concentration (Beitins *et al*, 1972) in cord plasma from infants delivered by elective Caesarean section is significantly lower than that in infants delivered vaginally. Indomethacin treatment in the late pregnancy results in a significant decrease in maternal levels of PRA and PA (Németh *et al*, in preparation).

Nevertheless, the mechanism by which prostaglandins induce renin release is not certain. Experimental evidence suggests that it can be ascribed to either prostaglandin-induced alterations in uteroplacental (Speroff, 1973 and 1975) and renal haemodynamics (Johnston *et al*, 1967) or to decreased pressor response to angiotensin II in peripheral vasculature (O'Brien *et al*, 1977). Also, it has been shown that prostaglandin E_2 reduces the rise in diastolic pressure in response to angiotensin II and the vascular sensitivity to angiotensin II is increased by intravenously administered indomethacin (O'Brien *et al*, 1977).

In conclusion, these data may be interpreted as indicating the possible role of prostaglandins in the increased activity of RAAS in the newborn. No claim is made that prostaglandins are the sole factors responsible for its development (Mott, 1975; Pipkin and Symonds, 1977). Further studies are needed to provide more direct information as to the importance of prostaglandins in the aetiology of neonatal overaction of the renin-angiotensin-aldosterone system (RAAS).

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EFFECT OF NORMAL PREGNANCY UPON THE GLYCOSYLATED HAEMOGLOBINS

BY

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Summary

In this cross-sectional study total glycosylated haemoglobin (Hb A_{Ia+b+c}) has been measured in 20 healthy non-pregnant women and in five groups of 20 healthy women at various times during pregnancy. A statistically significant decrease occurs in these minor haemoglobin fractions by about 20 weeks gestation and this reduced level is maintained throughout the rest of pregnancy. If diabetic patients are to have their control assessed during pregnancy by determination of these fractions this fact might be relevant.

ABOUT 90 per cent of the haemoglobin present in adults is Hb A (α_2 β_2), while Hb A_2 (α_2 δ_2) and Hb F (α_2 γ_2) comprise about 2·5 per cent and 0·5 per cent respectively. There are a number of minor components which can be separated from Hb A by chromatography on a cation exchange resin (Gabbay *et al*, 1977); these have been designated Hb A_{Ia-e} in the order in which they elute ahead of the major haemoglobin A fraction (Allen *et al*, 1958; Clegg and Schroeder, 1959).

Interest in three of these minor haemoglobins, Hb A_{Ia}, Hb A_{Ib} and Hb A_{Ic} has been stimulated by recent reports that their concentration in the peripheral circulation of diabetic patients may reflect long-term blood glucose control (Gabbay *et al*, 1977; Ditzer and Kjaergaard, 1978; Gonen *et al*, 1977; Koenig *et al*, 1976; Paulsen and Koury, 1976; Peterson *et al*, 1977; Trivelli *et al*, 1971) In normal, healthy adults Hb A_{Ia} and b form about 2 per cent of the total haemogllbin and Hb A_{Ic} about 5 per cent; they appear to result from the chemical condensation of Hb A and glucose, the glucose being linked to the N-terminal amino group of the β-chain. In diabetic patients these fractions can increase to about 3 per cent and 10 per cent respectively (Trivelli *et al*, 1971).

We wished to investigate whether the assay of these minor haemoglobin fractions would help in the clinical management of diabetic patients during pregnancy. But to answer this question we first needed to determine whether normal pregnancy in healthy women has any modifying influence on these glycosylated haemoglobins. One report (Schwartz *et al*, 1976) compared 15 healthy pregnant women of unspecified gestation with six normal, non-pregnant females and stated that the concentration of Hb A_{Ic} was increased by pregnancy. This seemed contrary to what might have been anticipated from the known decrease in fasting plasma glucose concentration during normal pregnancy (Lind *et al*, 1973) and the progressive increase in the total circulating red cell volume (Hytten and Lind, 1973).

In this paper we report that the concentration of total glycosylated haemoglobin has decreased to a statistically significant extent by the 20th week of gestation and that this lower level is maintained until term.

METHODS

One hundred women aged between 20 and 40 years were recruited as being in good general health, free of any family history of diabetes mellitus and, where pregnant, of having an uncomplicated antenatal period up to the time the blood sample was obtained. The women were divided into five groups of 20: non-pregnant, and pregnant at 9 to 13 weeks, 20 to 22 weeks, 26 to 28 weeks and 36 to 38 weeks gestation. A further group of 19 healthy women was investigated at about 38 weeks gestation; 12 had taken regular oral iron supplementation while 7 had never taken iron at any time during pregnancy.

The measurement of total glycosylated haemoglobin (Hb A_{Ia+b+c}) was as described by Kynoch and Lehmann (1977). Briefly, whole blood was haemolyzed using white saponin in potassium cyanide and applied to columns containing Bio-Rex 70 cation exchange resin mesh 200 to 400. The haemoglobins A_{Ia+b+c} are eluted into a 100 ml volumetric flask with 100 ml of a specific phosphate-cyanide buffer pH 6·8. The remainder of the haemoglobin (essentially HbA + HbA_2) is eluted into a second flask with 100 ml of a second buffer pH 6·4. Particular care was taken to maintain the correct pH at each stage of the determination; after covering the dry exchange resin with phosphate-cyanide buffer at pH 6·8 and allowing it to swell, the pH (now about 7·4) was adjusted to pH 6·8 again by adding normal hydrochloric acid drop by drop. Mixing by magnetic stirrer continued overnight after which the supernatant buffer was removed by suction and fresh buffer added. The pH was again checked regularly and returned to pH 6·8 while mixing for a further 24 hours. After aspirating and washing again with fresh buffer, columns (2×5 cm) were prepared in 20 ml plastic syringe barrels. The columns were then washed through with 150 to 200 ml of fresh buffer at pH 6·8 before use.

The pH of the prepared haemolysates of whole blood was also adjusted to pH 6·8 before being added to the column. After one run each column was washed overnight in fresh buffer before being used again. In this way clear separation of the glycosylated fractions from HbA was obtained and confirmed by acrylamide gel electrophoresis of these fractions. The need to adjust the pH is emphasized as it is not brought out in the original paper by Kynoch and Lehmann (1977).

This procedure does not clearly separate these glycosylated fractions from HbF and if it is suspected that a patient has more than the usual 0·8 per cent or so of fetal haemoglobin, the actual concentration ought to be specifically determined. The other minor variants Hb A_{Id+e} and Hb A_{GSSG} will also elute with the glycosylated haemoglobins but are quantitatively negligible in fresh blood.

Samples from 20 individuals were analysed (range 7·5 to 19·7 per cent) and the determinations repeated four days later. The standard deviation of the difference between repeat estimates was ±0·6 per cent; the values on the second occasion were about 0·3 per cent less than on the first occasion which may reflect slight differences in column characteristics from one occasion to another.

RESULTS

The percentages of glycosylated haemoglobin for individual patients together with the means and standard deviations for each group are given in Table I. There was no statistical difference between the non-pregnant women and those at 9 to 13 weeks gestation. By 20 to 22 weeks the percentage of Hb A_{Ia+b+c} was significantly lower than the non-pregnant value ($p < 0{\cdot}01$) as were the values at 26 to 28 weeks ($p < 0{\cdot}05$) and 36 to 38 weeks ($p < 0{\cdot}01$). However, the values at 20 to 22, 26 to 28 and 36 to 38 weeks gestation were not significantly different from each other. It thus appears that the pregnancy effect occurs prior to mid-pregnancy and is maintained thereafter.

Oral iron supplements given during normal pregnancy increases haemoglobin concentration, red cell count and MCV (Taylor and Lind, 1976) as well as total red cell mass (Hytten and Lind, 1973). To see if this in turn affected the percentage of glycosylated haemoglobin, the values at 36 to 38 weeks gestation from 12 women who had taken iron regularly throughout pregnancy were compared to those of 7 women who had never taken iron but no significant differences were found between the groups.

TABLE I

Percentage of glycosylated haemoglobin (Hb A_{Ia+b+c}) in non-pregnant women and in women at different times during pregnancy (20 patients in each group)

	Total glycosylated haemoglobin (per cent)				
		Gestation (weeks)			
	Non-pregnant	9 to 13	20 to 22	26 to 28	36 to 38
	5·7	4·2	5·2	4·6	5·0
	6·1	6·0	5·5	5·1	5·4
	6·2	6·2	5·9	5·2	5·5
	6·2	6·6	6·2	5·5	5·6
	6·6	6·6	6·3	5·6	5·7
	7·0	6·8	6·4	5·8	5·7
	7·1	6·8	6·6	5·8	5·8
	7·2	7·1	6·7	6·1	5·9
	7·2	7·5	6·7	6·2	6·0
	7·5	7·6	6·8	6·3	6·4
	7·5	7·7	6·8	6·7	6·5
	7·7	7·7	7·0	6·8	6·7
	7·7	8·0	7·0	7·2	7·1
	7·9	8·0	7·0	7·3	7·1
	8·1	8·1	7·1	7·5	7·2
	8·2	8·2	7·2	7·6	7·4
	8·4	8·4	7·2	8·0	7·5
	8·7	8·4	7·3	8·1	7·7
	8·8	8·5	7·6	8·6	8·3
	9·0	8·5	7·9	8·9	8·5
Mean	7·44	7·35	6·72	6·65	6·55
± SD	0·95	1·08	0·67	1·22	1·01

DISCUSSION

The slow chemical reaction of glycosylation occurs throughout the lifespan of the erythrocyte and the rate of formation of the major fraction (Hb A_{Ic}) appears to be directly proportional to the time-averaged concentrations of glucose within the erythrocyte (Bunn *et al*, 1976). Thus in normal individuals with average blood glucose values over 24 hours about 6 to 7 per cent of haemoglobin is glycosylated; in diabetics with consistently elevated blood glucose concentrations levels of 15 per cent or more may be achieved (Paulsen and Koury, 1976).

By 10 weeks gestation the fasting blood glucose concentration is about 5 mg/dl (0·3 mmol/l) lower than in non-pregnant women and the reduced level is maintained for the rest of pregnancy (Lind *et al*, 1973). Most pregnant women return their blood glucose levels to the fasting concentration within two hours of glucose ingestion (Lind *et al*, 1973) and, with meals about four times per day, the glucose level is going to be around the lower fasting value for about 16 hours out of every 24. New erythrocytes formed from 10 weeks gestation onward will therefore be exposed to a lower time-averaged glucose concentration than those of non-pregnant women and the degree of glycosylation should therefore be less.

The red cell volume increases progressively throughout pregnancy; women not taking iron have an incremental increase of about 250 ml (18 per cent) while those who take regular iron supplements may have increases of 400 ml (30 per cent) or more (Hytten and Lind, 1973). Fitzgibbons *et al* (1976) have reported that the Hb A_{Ic} content of the 10 per cent youngest erythrocytes in 15 normal non-pregnant subjects was 3·1±0·8 per cent while for the 10 per cent oldest cells the value had increased to 6·0±1·1 per cent. It would therefore be expected that the influx of new young erythrocytes causing the progressive increase in red cell mass during pregnancy would 'dilute' the total amount of glycosylated haemoglobin. The present data confirm that this occurs; however, as new cells would eventually glycosylate, it might be anticipated that by term the percentage of glycosylated haemoglobin would be beginning to increase and this is not shown by our data. This could be accounted for if the maximum glycosylation occurring as the cells aged was less than in non-pregnant subjects. A small reduction in the maximum percentage glycosylation achieved would account for the flattening of response after 20 weeks gestation which we have found. The fact that women near term who regularly took iron supplementation had the same percentage of glycosylated haemoglobin as those who did not take iron also argues for the general reduction in the degree of glycosylation. The former group of women would have a greater number of circulating erythrocytes as a result of the iron stimulation (Taylor and Lind, 1976) and any 'dilutional' effect of increased numbers of young erythrocytes should have produced lower values for their glycosylated fraction.

Our conclusions are drawn from a cross-sectional study and it cannot be taken for granted that individual patients will necessarily show this trend. Indeed Table I shows that at each stage of gestation there are some individuals who have total glycosylated haemoglobin values of about 8 per cent. However, a consideration of the known physiological adaptations during pregnancy would suggest that the glycosylated haemoglobin fraction should decrease somewhat and our data support this. If diabetic patients are to have their control assessed during pregnancy by determination of these fractions the physician and obstetrician should be aware that some fall in concentration may be reflecting this pregnancy effect as well as a possible improvement in their clinical control.

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THE SIGNIFICANCE OF ABNORMAL GLUCOSE TOLERANCE (HYPERGLYCAEMIA AND HYPOGLYCAEMIA) IN PREGNANCY*

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Summary

Maternal hypoglycaemia (plasma glucose below 5th centile) had a highly significant association with fetal growth retardation, and perinatal mortality was significantly increased in the presence of both hypoglycaemia and hyperglycaemia (plasma glucose above 95th centile) when pregnancy outcome was analyzed in 5000 consecutive patients who had a glucose tolerance test performed during the third trimester of pregnancy. This study confirms the significance of abnormal glucose tolerance as a causative factor of feto-placental dysfunction. The flat glucose tolerance test pattern had no significance beyond the presence of associated hypoglycaemia, but reactive hypoglycaemia, and persistent abnormalities of plasma glucose levels during the test, were associated with higher incidences of complicated outcome. Hypertonic dextrose therapy administered to the patient with persistently subnormal urinary oestriol excretion was less likely to cause a favourable response in oestriol excretion if glucose tolerance was abnormal, perhaps because the adverse influences of abnormal glucose tolerance were not reversible by the third trimester of pregnancy. Hypoglycaemia and hyperglycaemia, additional to diabetes mellitus, are significant factors in the aetiology and diagnosis of abnormal pregnancy, and point to the need to investigate therapeutic measures.

FETAL growth is dependent upon the availability of essential metabolites such as glucose, amino acids and free fatty acids as well as minerals and vitamins supplied from the mother via the placenta.

Glucose is the simplest energy substrate, and it would be logical to expect that a relation between glucose supply and demand must be important in fetal growth and development.

The purpose of this study was to evaluate this possibility by the prospective analysis of a consecutive series of 7000 patients who had routine glucose tolerance tests and urinary oestriol estimations in late pregnancy to see if there was any association between abnormalities of glucose tolerance and pregnancy complications.

METHODS

From June 1971 glucose tolerance was tested routinely at 32 to 34 weeks gestation on all antenatal patients attending the Mercy Maternity Hospital Melbourne.

Patients continued their normal diet and were instructed to fast from 2200 hours on the night before the test; at 0900 hours they began a 50 g three-hour oral glucose tolerance test. Before the test and at 1, 2 and 3 hours after the glucose load, capillary blood was obtained from a finger prick, collected into a heparinized tube and immediately centrifuged. Plasma glucose was measured immediately by the glucose oxidase method using a Beckman glucose analyser (Beckman Instruments, Inc, Fullerton, Ca, USA, 92634). This method utilizes 10 μl of plasma and measures only true glucose, correlating closely with other methods of analysis (Stevens, 1971), with a coefficient of variation of ± 2 per cent on replicate sampling. Quality control was checked every third or fourth analysis by calibration against the 150 mg/100 ml glucose standard supplied by the manufacturer.

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* Prepared from the William Blair-Bell Memorial Lecture delivered at the Royal College of Obstetricians and Gynaecologists, 1st December, 1976.

Patients excluded were those with multiple pregnancies. Otherwise the series was consecutive but, because of the period of gestation at which glucose tolerance was tested, did not include those delivered before 32 weeks, and those admitted unbooked in labour or with emergency obstetric complications which precluded an opportunity to perform the glucose tolerance test.

Urinary oestriol excretion was measured routinely on all patients at 30 and 36 weeks of gestation and repeated when indicated clinically or when excretion was low, that is, below a line joining 8 mg/24 hours at 30 weeks and 12 mg/24 hours at 40 weeks and thereafter.

An infant was classified as small-for-dates when birth weight was below the 10th centile and large-for-dates when above the 90th centile according to standards for infants born in this community (Kitchen, 1968). The 10th and 90th centiles used for defining small and large-for-dates placentae were those previously established for this community (Ratten *et al*, 1974).

All infants were carefully examined within 48 hours of birth for the presence of malformations, this study being part of a prospective investigation into the incidence of major and minor malformations in the hospital community.

Perinatal mortality included any infant stillborn after 28 weeks gestation, and deaths occurring in liveborn infants within 28 days of birth (neonatal deaths).

INITIAL STUDIES

In the first 2000 consecutive patients investigated, 1000 of whom were public and 1000 private patients, the 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles for plasma glucose at each of the four readings of the test were determined (Table I). A normal range for all patients, based on the 5th and 95th centiles was derived from these figures (Table II). Hypoglycaemia was diagnosed when the plasma glucose equalled or was less than the lower limits of normal on one or more of the four readings obtained; hyperglycaemia when the plasma glucose equalled or exceeded the upper limits of normal on one or more occasion, and normoglycaemia when all four plasma glucose levels fell between the lower and upper limits of normal. Gestational

TABLE I

Glucose tolerance test centiles (1000 public and 1000 private patients)

Centile	Plasma glucose															
	Fasting				One hour				Two hours				Three hours			
	Public		Private		Public		Private		Public		Private		Public		Private	
	mg/dl	mmol/l	mg/dl	mmol/l	mg/dl	mmol/l	mg/dl	mmol/l	mg/dl	mmol/l	mg/dl	mmol/l	mg/dl	mmol/l	mg/dl	mmol /l
97th	96	5·3	93	5·2	174	9·7	178	9·9	138	7·7	139	7·7	110	6·1	110	6·1
95th	93	5·2	91	5·0	165	9·2	172	9·5	128	7·1	132	7·3	103	5·7	106	5·9
90th	88	4·9	87	4·8	154	8·5	162	9·0	120	6·7	122	6·8	95	5·3	97	5·4
50th	76	4·2	78	4·3	123	6·8	127	7·0	95	5·3	101	5·6	70	3·9	71	3·9
10th	65	3·6	68	3·8	95	5·3	101	5·6	74	4·1	80	4·4	58	3·2	58	3·2
5th	61	3·4	65	3·6	88	4·9	95	5·3	68	3·8	74	4·1	55	3·0	56	3·1
3rd	58	3·2	64	3·5	83	4·6	91	5·0	63	3·5	70	3·9	53	2·9	53	2·9

TABLE II

Upper and lower limits of normal glucose tolerance

Definition	Plasma glucose (mg/dl)			
	Fasting	One hour	Two hours	Three hours
Upper limits of normal (hyperglycaemia)	95	170	130	105
Lower limits of normal (hypoglycaemia)	65	90	70	55
Gestational diabetes:	2 hour plasma glucose level 140 mg/100 ml or above and maximum level at any time 180 mg/100 ml or above			

diabetes was diagnosed when the plasma glucose was 140 mg/100 ml or above at the two-hour reading and 180 mg/100 ml or above at any time during the three-hour glucose tolerance test. Patients whose glucose tolerance test had both a hypoglycaemic and a hyperglycaemic level were included in the results of both categories.

Hypoglycaemia was found to be associated with fetal and placental growth retardation, whilst hyperglycaemia was associated with increased placental weight but not with increased fetal birth weight (Abell *et al*, 1976*a*). Perinatal mortality was closely related to maternal glucose tolerance being significantly increased in the presence of either hypoglycaemia or hyperglycaemia. Subnormal urinary oestriol excretion occurred more frequently in pregnancies complicated by abnormal glucose tolerance, but there was no apparent association between abnormal glucose tolerance and major fetal malformations.

An unexpected finding was that hyperglycaemia at the one-hour reading was associated with an increased incidence of fetal growth retardation (Abell and Beischer, 1975*a*). Intra-uterine growth retardation in the presence of hypoglycaemia was most common when this occurred at the three-hour reading, that is, when the glucose tolerance test demonstrated reactive hypoglycaemia (Abell and Beischer, 1975*b*).

The association of abnormal glucose tolerance with pregnancy outcome when oestriol excretion was subnormal was studied in 611 consecutive patients who had low oestriol excretion (Abell and Beischer, 1975*a*). In 265, oestriol excretion was persistently subnormal, that is, low on two or more consecutive occasions, and in these patients the perinatal mortality rate was 57 per thousand. Normoglycaemia was associated with a perinatal mortality rate of 17, whereas in the presence of hypoglycaemia the rate was 148 (P <0·001) and when there was hyperglycaemia it was 125 (P <0·01). Small-for-dates babies occurred in 46·3 per cent of pregnancies complicated by hypoglycaemia and persistently subnormal urinary oestriol excretion.

A total of 5000 consecutive patients who had a glucose tolerance test during pregnancy has now been studied and these observations were confirmed (Abell and Beischer, 1976*b*).

PRESENT STUDIES AND DISCUSSION

The present studies were an endeavour to further understand the significance of abnormal glucose tolerance (hypoglycaemia and hyperglycaemia) in pregnancy.

Fetal size

Although in our initial studies there was no demonstrable association between hyperglycaemia and babies weighing more than 4000 g (Macafee *et al*, 1974; Abell and Beischer, 1976*a*) or large-for-dates babies (Abell *et al*, 1976*a*), a study was undertaken to evaluate glucose tolerance patterns in 59 pregnancies where the baby at delivery weighed 4540 g (10 lb) or more. Hyperglycaemia was present in 15 (25 per cent) of these patients. None of the glucose tolerance tests was diagnostic of diabetes, and 76 per cent of the multiparous patients in this group had previously been delivered of a baby weighing 3630 g (8 lb) or more. Although increasing hyperglycaemia is not linearly related to increasing fetal size (Abell and Beischer, 1976*a*), hyperglycaemia may be associated with an increased incidence of excessively large babies. This small group of patients may progress to diabetes in later life (Kritzer, 1952; Mickal *et al*, 1966; O'Sullivan *et al*, 1971).

Perhaps the actual cause of the large baby in pre-diabetes and in diabetes is not glucose alone. Administration of insulin in the pre-diabetic suppresses excessive fetal growth (O'Sullivan *et al*, 1971) but has no influence on fetal size in the diabetic (Karlsson and Kjellmer, 1972). This infers a close aetiological relationship between diabetes and fetal growth. As the aetiology of diabetes mellitus is incompletely understood so the stimulus to excessive fetal growth also remains unanswered.

Major fetal malformations

There were 247 consecutive infants with major malformations whose mothers had a glucose tolerance test during the pregnancy. Hypoglycaemia was diagnosed in 15·8 per cent and hyperglycaemia in 14·6 per cent. The incidence of hyperglycaemia did not significantly differ from that in the community survey of 5000 patients (11·7 per cent; Abell and Beischer, 1976*b*).

In two of these pregnancies the mother was known to be diabetic before pregnancy, and only three were diagnosed as having gestational diabetes on routine glucose tolerance testing. The incidence of maternal diabetes was therefore 2·0 per cent (5 of 247) in pregnancies complicated by a major fetal malformation. This compares with the incidence of maternal diabetes and gestational diabetes of 0·7 per cent in the earlier study of 5000 patients (Abell and Beischer, 1976*b*): in which study 5 babies born of the 36 diabetic pregnancies (13·9 per cent) had a major malformation. Although major fetal malformations are more common in pregnancies complicated by diabetes mellitus, these patients represent a numerically small proportion of the population, so that screening for diabetes in mothers delivered of a malformed infant would have a detection rate not sigficantly higher than that obtained by testing the entire pregnant population.

We have found an association between hypoglycaemia and malformations of the genital organs and club foot, and between hyperglycaemia and malformations of the central nervous system (Drew *et al*, 1978). These findings are consistent with the reported positive association of spinal abnormalities with diabetes and negative association of hypospadias found in a reviewed series of 340 malformed infants born of 7101 diabetic pregnancies (Kučera, 1971). It is therefore suggested that glucose itself is the relevant aetiological factor in certain fetal malformations.

During pregnancy complicated by fetal malformation, the incidences of fetal growth retardation and subnormal urinary oestriol excretion are approximately double the average for the population (Macafee *et al*, 1972). As the preliminary studies had also revealed associations between abnormal glucose tolerance and increased incidences of growth retardation and low oestriol excretion (Abell *et al*, 1976*a*), the relation between major fetal malformations and abnormal glucose tolerance could be incidental as much as causal. The inter-relations between growth retardation, subnormal oestriol excretion and fetal malformations are therefore associated with abnormal maternal glucose tolerance although undefined causal factors may also be present.

Perinatal mortality

There were 90 patients who had a perinatal death and who had had a glucose tolerance test before delivery. Of these infants 56 were stillborn and 34 died in the neonatal period. Twenty deaths were associated with maternal hypoglycaemia, 49 with normoglycaemia and 23 with hyperglycaemia. The association between abnormal glucose tolerance and perinatal mortality was highly significant ($P < 0·001$) when compared to the incidence of abnormal glucose tolerance in the community survey of 5000 patients (Abell and Beischer, 1976*b*).

The causes of death were studied in relation to the maternal glucose tolerance pattern. Abruptio placentae and respiratory distress syndrome may have some association with maternal hypoglycaemia and this observation requires further analysis in a larger group of patients. There were 31 hypoxic stillbirths in which there was a trend towards intrauterine growth retardation and persistently low urinary oestriol excretion associated with hypoglycaemia or hyperglycaemia.

The cause of perinatal death in diabetic pregnancies is frequently unknown (Macourt, 1974). For this reason management of the pregnant diabetic has been basically empirical with emphasis on adequate control of the diabetes and early delivery. A policy of allowing the pregnancy to proceed if oestriol excretion is normal and there are no other complicating obstetric or medical factors has been advocated

(Schwarz *et al*, 1969; Rivlin *et al*, 1970; Schwarz and Fields, 1971), but caution has been expressed regarding this form of management in that normal oestriol excretion may not exclude the possibility of impending fetal death (Evans *et al*, 1972; Masson and Sutherland, 1973; Ostergard, 1973). Medical treatment of the diabetes remains the most important facet of the care of these patients during pregnancy (Posner *et al*, 1971; O'Sullivan *et al*, 1974). Perinatal mortality has been shown to be impressively reduced when mean maternal glucose has been controlled to within normal limits (Karlsson and Kjellmer, 1972) and it would appear that intensive control of maternal glucose to normoglycaemic levels should be the fundamental aim of management (Victor, 1974). The finding in this study that perinatal mortality was elevated when there was maternal hyperglycaemia supports this principle. Whether the patients in the present study with hyperglycaemia have a higher risk of becoming diabetic in later life (Mestman *et al*, 1972) remains to be evaluated by long-term follow-up studies.

The observation that hypoglycaemia was associated with an increased perinatal mortality rate stresses the hazards of all forms of abnormal maternal glucose tolerance to fetal wellbeing, and again specifically demonstrates the advantages of normoglycaemia.

Patterns of abnormal glucose tolerance

A particular pattern of glucose tolerance has been described in association with incipient diabetes mellitus, in which the plasma glucose rises to high levels then falls precipitously to cause associated symptomatic hypoglycaemia (Seltzer *et al*, 1956). The hypoglycaemia is believed to be due to an exaggerated response in insulin secretion and to precede total pancreatic exhaustion and hence diabetes. A pattern of glucose tolerance when there was a hyperglycaemic response at the one or two-hour reading of the test, followed by hypoglycaemia at the two or three-hour reading was observed in 16 patients of the 5000 surveyed. The pregnancy outcome in these patients is shown in Table III. There was a trend towards a higher incidence of all pregnancy complications studied although the numbers were too small for statistical confirmation. Such a pattern of abnormal glucose tolerance may prove to be significant.

A 'flat' glucose tolerance curve has been regarded as a variant of normal and is presumably due to a relative excess of insulin secretion (Das Gupta and Whitehouse, 1971; Nolan *et al*, 1972). Such a pattern, where increments or decrements between consecutive readings did not exceed 20 mg/100 ml plasma glucose was seen in 86 of 2000 (4·3 per cent) consecutive patients. The pregnancy outcome in these patients is shown in Table IV. There was no significant alteration in incidences of the pregnancy complications studied. Any obstetric significance associated with a 'flat' glucose tolerance curve would be identified by the

TABLE III

Pregnancy outcome when glucose tolerance test pattern showed reactionary hypoglycaemia suggestive of early diabetes

Pregnancy complication	Incidence of complication No.	 Per cent
Number of patients	16 of 5000 surveyed	
Subnormal oestriol excretion	4	25
Small-for-dates baby	3	19
Large-for-dates baby	3	19
Small-for-dates placenta	5	32
Large-for-dates placenta	4	25
Major fetal malformations	3	19
Perinatal mortality	1	6

TABLE IV

Pregnancy outcome when glucose tolerance test pattern was flat

Pregnancy complication	Incidence of complication No.	 Per cent
Number of patients	86 of 2000 surveyed	
Subnormal oestriol excretion	7	8·1
Small-for-dates baby	8	9·3
Large-for-dates baby	4	4·7
Small-for-dates placenta	7	8·1
Large-for-dates placenta	9	10·5
Major fetal malformations	5	5·8
Perinatal mortality	3	3·5

occurrence of hypoglycaemia rather than the pattern of the curve itself.

The classifications of abnormal glucose tolerance were subdivided into two principal groups according to whether the abnormality of plasma glucose was confined to only one of the four readings obtained (Group 1) or was present on more than one occasion during the test (Group 2). Hyperglycaemia of the second group also included those patients diagnosed as having gestational diabetes and those known to be diabetic before pregnancy. The pregnancy outcome according to the group of hypoglycaemia and hyperglycaemia seen in 5000 consecutive patients is shown in Table V. Persistent hypoglycaemia (Group 2) identified higher incidences of subnormal urinary oestriol excretion (P <0·05), small-for-dates babies and placentae, and perinatal mortality when compared to the outcome in those pregnancies with Group 1 hypoglycaemia. Persistent hyperglycaemia (Group 2) was associated with higher incidences of subnormal urinary oestriol excretion (P <0·05), major fetal malformations and perinatal mortality, but not of large-for-dates babies, than those seen in patients with Group 1 hyperglycaemia.

Dextrose therapy

It might be expected that pregnancy outcome could be improved by control of maternal glucose levels, exemplified by the improved results achieved by careful management of the pregnant diabetic. Therapeutic possibilities in the hypoglycaemic patient would appear obvious.

Routine measurement of urinary oestriol excretion in patients at this hospital has shown that this investigation significantly detected the pregnancy at risk of intrauterine growth retardation and perinatal mortality (Beischer *et al*, 1977). Persistently low oestriol excretion occurred in approximately 3 per cent of patients. For four years approximately two-thirds of patients with persistently low urinary oestriol excretion have been eligible for treatment with hypertonic (25 per cent) dextrose if the fetus was judged too immature to survive if born alive and there was no maternal contraindication such as pre-eclampsia to this type of conservative treatment (Beischer and O'Sullivan, 1972; Beischer *et al*, 1974). Therapy in this high-risk group of patients was regarded as successful only if the urinary oestriol excretion returned to, and remained at, normal levels. Table VI shows the response to therapy in the first 169 patients according to the pattern of glucose tolerance. Patients with abnormal glucose tolerance were less likely to respond successfully, particularly when there was hypoglycaemia (P <0·05). Table VII depicts the pregnancy outcome in those patients in whom hypertonic dextrose therapy had failed. Hypoglycaemia and hyperglycaemia were asso-

TABLE V

Pregnancy outcome according to group of hypoglycaemia or hyperglycaemia

Pregnancy complication	Glucose tolerance									
	Hypoglycaemia				Normoglycaemia		Hyperglycaemia			
	Group 1		Group 2				Group 1		Group 2	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Number of patients	640		98		3716		466		120	
Subnormal oestriol excretion	98	15·3	24	24·5*	479	12·9	63	15·6	29	24·2*
Small-for-dates baby	82	12·8	18	18·4	281	7·6	43	9·2	11	9·2
Large-for-dates baby	55	8·6	4	4·1	339	9·1	56	12·0	11	9·2
Small-for-dates placenta	100	15·6	22	22·4	433	11·7	49	10·5	11	9·2
Large-for-dates placenta	38	5·9	9	9·2	318	8·6	62	13·3	10	8·3
Major fetal malformations	30	4·7	5	5·1	123	3·3	19	4·1	9	7·5
Perinatal mortality	12	1·9	3	3·1	36	1·0	13	2·8	5	4·2

Significance of difference between Group 1 and Group 2; * P <0·05

TABLE VI

Response to hypertonic dextrose therapy according to maternal glucose tolerance pattern

Glucose tolerance	Number of patients	Urinary oestriol response			
		Failed		Successful	
		No.	Per cent	No.	Per cent
Total	169	69	40·8	100	59·2
Hypoglycaemia	31	18	58·1	13	41·9*
Normoglycaemia	120	43	35·8	77	64·2
Hyperglycaemia	20	9	45·0	11	55·0

* P <0·05

TABLE VII

Pregnancy outcome in patients who did not respond to hypertonic dextrose therapy, according to maternal glucose tolerance pattern

Pregnancy complication	Total patients		Glucose tolerance					
			Hypoglycaemia		Normoglycaemia		Hyperglycaemia	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Number of patients	69		18		43		9	
Small-for-dates baby	32	46·4	12	66·7*	16	37·2	5	55·6
Large-for-dates baby	1	1·4	—		1	2·3	—	
Small-for-dates placenta	24	34·8	11	61·1**	11	25·6	3	33·3
Large-for-dates placenta	1	1·4	—		—		1	11·1
Major malformations	6	8·7	1	5·6	4	9·3	1	11·1
Perinatal mortality	9	13·0	5	27·8	3	7·0	2	22·2

* P <0·05 ** P <0·01

ciated with extremely high perinatal mortality rates, and a considerable excess of poor fetal growth.

The fact that those patients with hypoglycaemia responded poorest as a group may be due to the effects of hypoglycaemia being irreversible by the time treatment was commenced. This would suggest that the adverse influences of abnormal glucose tolerance were early, possibly on the placenta, and not correctable in the third trimester.

The placental transfer of glucose may be dependent upon maternal uterine and placental blood flow (Joyce and Young, 1974; Krauer *et al*, 1973), and to this end studies are currently in progress evaluating the use of beta-mimetic drugs in increasing uterine blood flow and inducing uterine relaxation to improve fetal nutrition and welfare in pregnancies when oestriol excretion is low.

CONCLUSIONS

Associations between abnormal glucose tolerance, intrauterine fetal growth retardation and subnormal urinary oestriol excretion have been repeatedly observed in this study. It is concluded that abnormal maternal glucose tolerance, hypoglycaemia and hyperglycaemia, additional to diabetes mellitus, is a significant parameter in the consideration of abnormal pregnancy, and its management. Whereas hypoglycaemia may be the primary causative factor of feto-placental dysfunction, the clinical associations with hyperglycaemia are more difficult to explain, and probably some additional diabetogenic factor is implicated. The most important finding in this study was that hypoglycaemia in pregnancy is a significant cause of perinatal mortality and fetal growth retardation (Abell *et al*, 1976*b*).

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PROTEASE INHIBITORS IN SERUM AND AMNIOTIC FLUID DURING PREGNANCY

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Summary

Antitryptic and antichymotryptic activities in amniotic fluid and maternal serum were measured at various stages of pregnancy using the caseinolytic assay method for proteases. The inhibitory activities increased up to 20 weeks in amniotic fluid and remained in the same range up to 30 weeks; a sharp fall was observed at term. In serum, there was a gradual rise of activities with peak values around 30 weeks. The decrease with further advance of pregnancy was not as sharp as in amniotic fluid. The ratios of antitryptic to antichymotryptic activities remained fairly constant (1·54±0·07) in amniotic fluid throughout pregnancy. In maternal serum the ratio varied over a range of 1·28 to 2·67 and the increase in antichymotryptic activity was relatively greater between 16 and 30 weeks resulting in a lower ratio between the two activities. Amniotic fluid was found to contain a higher proportion of a heat stable inhibitor compared to serum and its contribution to total antitryptic activity varied from 8·7 to 15·6 per cent.

HUMAN serum contains a variety of proteins which are capable of inhibiting trypsin, chymotrypsin and other related proteolytic enzymes (Heimburger *et al*, 1971). Quantitatively the most important of these are α–1 antitrypsin and α–2 macroglobulin (Travis *et al*, 1974); they are known to be glycoproteins. Most of the inhibitory activity of serum against trypsin is due to α–1 antitrypsin (Talamo, 1971), and increased levels have been observed in pregnancy (Ganrot and Bjerre, 1967). The protease inhibitors are considered to play a protective role against proteolytic action of pancreatic and intracellular proteases.

Giltin and Perricelli (1970) found α–1 antitrypsin in amniotic fluid and Sutcliffe and Brock (1973) have shown that it is one of the major proteins. Studies on the changes in the levels of α–1 antitrypsin in amniotic fluid by immunochemical techniques have given conflicting results. Guibaud *et al* (1975) who estimated α–1 antitrypsin levels in amniotic fluid by a radioimmunodiffusion method concluded that there was no change in level up to 30 weeks of pregnancy, after which there was a fall. The work of Giltin and Perricelli (1970), Monaghan *et al* (1973) and Evans *et al* (1975) indicated no systematic change with gestation. On the other hand, Sutcliffe and Brock (1973) using an immunoprecipitation technique concluded that amniotic fluid antitrypsin concentration increased up to 20 weeks, then remained constant up to 30 weeks, finally falling rapidly in later pregnancy. While the work of Guibaud *et al* (1975) showed wide individual variation of level (greater than 10-fold in specific age groups), Monaghan *et al* (1973) reported much less individual variation.

In the present study we have measured the levels of antitryptic and antichymotryptic activity during pregnancy in serum and amniotic fluid by a colorimetric method. Presence of a new inhibitor in amniotic fluid, which is different from α–1 antitrypsin and which is heat stable, is demonstrated for the first time.

MATERIALS AND METHODS

Seventy-three samples of amniotic fluid were analyzed in these studies. The samples between 12 to 30 weeks gestation were mostly obtained during medical termination of pregnancy. The samples at full term were obtained before delivery. Blood samples from pregnant women were collected at various stages of gestation.

Bovine trypsin (twice crystallized) and bovine chymotrypsin (thrice crystallized) were obtained from Worthington Biochemical Corporation, USA; DEAE–cellulose from Bio-Rad Chemicals, USA. All other reagents were of analytical grade. Active site titrations of trypsin and chymotrypsin were performed as described by Kezdy and Kaiser (1974) using p-nitrophenyl acetate as substrate to determine the purity of the enzyme. The trypsin and chymotrypsin samples used were found to have 60 per cent and 70 per cent activity respectively, and the enzyme activities and inhibitory activities were correspondingly corrected.

Antitryptic and antichymotryptic activities in amniotic fluid were determined as follows. Suitable aliquots of the filtered amniotic fluid (10 to 50 μl) were preincubated with 0·2 ml of trypsin (3·6 μg) or chymotrypsin (3·5 μg) in the presence of 20 μmol of phosphate buffer, pH 7·6, in a total volume of 1·0 ml at 37°C for 10 minutes. One ml of 2 per cent casein solution containing 100 μmol of phosphate buffer, pH 7·6, was added. After 20 minutes incubation at 37°C, 3·0 ml of 5 per cent trichloroacetic acid was added to arrest enzyme action. The precipitate was sedimented by centrifugation at 2500 × g for 10 minutes, after standing for 30 minutes. One ml of the clear supernatant was analyzed for soluble protein fragments by the method of Lowry *et al* (1951) using bovine serum albumin as standard. Enzyme controls without amniotic fluid were run simultaneously. Difference in the absorbance between test and control was a measure of the inhibitory activity. Inhibitory activities in serum were measured similarly using aliquots of 50 to 200 μl of 100-times diluted serum. Aliquots of fluids were chosen such that inhibition was in the range of 30 to 60 per cent of the enzyme activity. One unit of inhibitory activity is equal to 1 mg of the enzyme (trypsin or chymotrypsin as the case may be) inhibited under the conditions of assay. The inhibitory activities as determined by this method were found to vary within a range of ±5 per cent.

Protein in amniotic fluid was determined by the Biuret method (Reinhold, 1953).

Heat stable trypsin inhibitor in serum and amniotic fluid were separated and assayed using the method of Proksch and Routh (1972) for isolation of urinary heat-stable trypsin inhibitor. Aliquots of 0·1 ml of 10 times diluted serum or 1·0 ml of diluted amniotic fluid was applied to a DEAE-cellulose column (Bed volume 1·0 ml) equilibrated with 0·02 M phosphate buffer pH 7·0. The column was eluted with 8 ml of 0·02 M phosphate buffer containing 0·12 M NaCl. The α–1 antitrypsin and other heat labile inhibitors were eluted in this fraction. Heat-stable trypsin inhibitor was eluted with 0·02 M phosphate buffer containing 0·3 M NaCl. This fraction was completely stable to heat treatment at 80°C for 10 minutes whereas, the earlier fraction was labile.

RESULTS

Antitryptic activity was determined in 73 samples of amniotic fluid and the values as a function of gestational period are presented in Figure 1. Antichymotryptic activities are presented similarly in Figure 2. In Table I, the data on antitryptic activity, antichymotryptic activity and protein in amniotic fluid at different gestational periods are summarized. In the early stages of pregnancy, the levels of antitryptic activity and antichymotryptic activity were low. There was a gradual increase in the values up to

20 weeks. However, no significant differences in the levels of the inhibitors was noticed during the gestation period of 20 to 30 weeks. At term, both the levels of antitryptic and antichymotryptic activities were found to be low, indicating a sharp fall in the second half of the third trimester. The changes in inhibitory activities in amniotic fluid during pregnancy roughly paralleled the changes in protein content. The ratio of antitryptic activity to antichymotryptic activity varied within a very narrow range of 1·43 to 1·68 (mean ± SD: 1·54 ± 0·07).

Changes in the levels of antitryptic and antichymotryptic activities in serum during pregnancy were examined in a total of 51 cases. Seventeen non-pregnant women aged 20 to 40 years served as controls. Changes in serum antitrypsin levels and antichymotrypsin levels as functions of gestational age are presented in Figures 3 and 4 respectively. In 33 out of 51 pregnancy serum samples, antitryptic activity was found to be elevated; antichymotrypsin levels were high in 46 out of 51 samples. The values which were in the normal range were

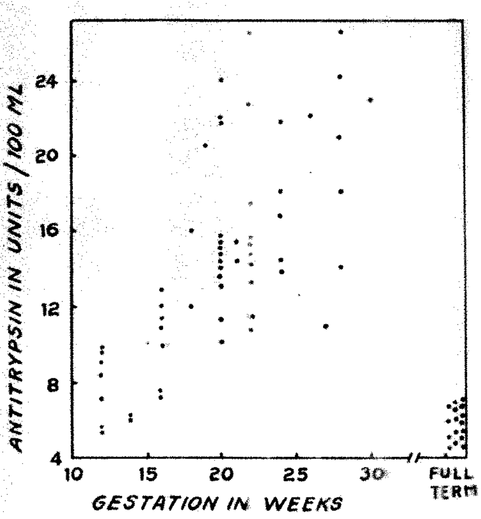

FIG. 1

Antitrypsin levels in amniotic fluid at different gestational periods.

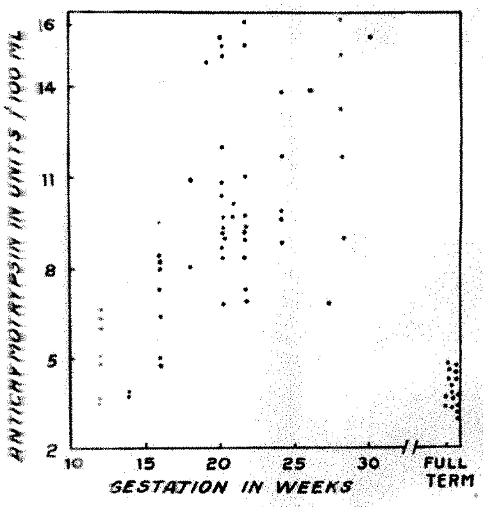

FIG. 2

Antichymotrypsin levels in amniotic fluid at different gestational periods.

TABLE I

Antitryptic, antichymotryptic activities and protein in amniotic fluid

Gestation (weeks)	No. of samples	Antitryptic activity (units/dl) Mean ± SD	Antichymotryptic activity (units/dl) Mean ± SD	Protein (mg/dl) Mean ± SD
12–14	9	7·42 ± 1·75	4·81 ± 1·24	333·0 ± 62·4
16–18	9	11·10 ± 2·48	7·37 ± 1·94	417·4 ± 85·3
20–21	16	15·50 ± 3·65	9·99 ± 2·34	563·9 ± 137·2
22–24	15	16·32 ± 4·47	10·48 ± 2·76	596·3 ± 118·3
26–30	8	19·88 ± 5·25	12·66 ± 3·30	557·3 ± 105·2
Full term	16	5·85 ± 0·75	3·94 ± 0·54	268·4 ± 83·1

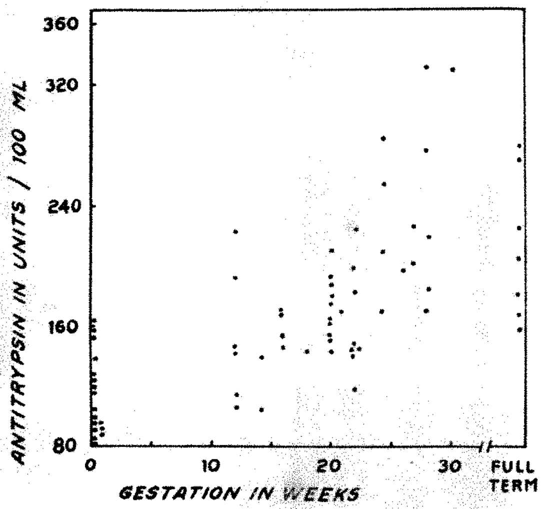

FIG. 3

Antitrypsin levels in serum at different gestational periods.

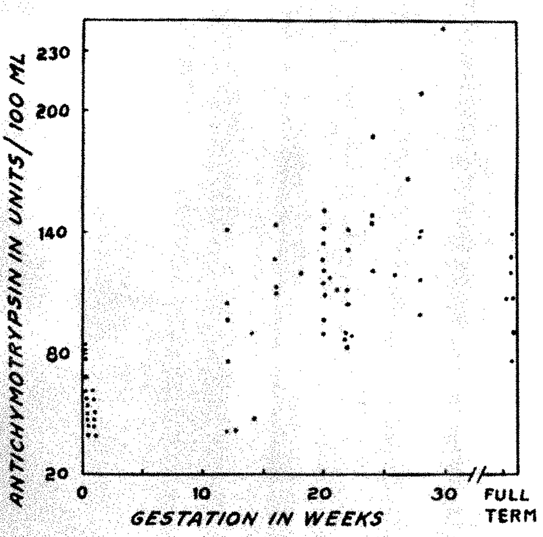

FIG. 4

Antichymotrypsin levels in serum at different gestational periods.

mostly in subjects in the early stages of pregnancy. There was a gradual increase of both antitryptic and antichymotryptic activities reaching maximal values at 26 to 30 weeks gestation. However, antichymotrypsin levels were found to increase more between 16 to 18 weeks than did antitrypsin levels. At term, the average values of both were slightly lower than those at 26 to 36 weeks. The levels of antitryptic activity and antichymotryptic activity in serum during pregnancy and in control subjects are shown in Table II.

TABLE II

Antitryptic and antichymotryptic activities in serum

Gestation (weeks)	No. of samples	Antitryptic activity (units/dl) Mean ± SD	Antichymotryptic activity (units/dl) Mean ± SD
Controls	17	115·7±28·8	58·5±15·1
12–14	8	145·7±41·6	79·9±35·2
16–18	5	156·1±10·4	122·4±14·0
20–21	11	171·8±20·2	119·5±17·8
22–24	12	183·5±51·2	120·1±30·9
26–30	8	238·9±63·1	153·3±48·4
Full term	7	211·1±48·1	109·0±22·6

TABLE III

Ratio of antitryptic activity to antichymotryptic activity in serum

Gestation (weeks)	No. of samples	Ratio Mean ± SD
Controls	17	2·01±0·16
12–14	8	1·97±0·47
16–18	5	1·28±0·12
20–30	31	1·53±0·22
Full term	7	1·94±0·28

Unlike amniotic fluid, the ratio of the two activities in serum varied over a wide range, from 1·28 to 2·67, during pregnancy (Table III). In control subjects, and in early and late pregnancy, the ratio was close to 2·0, whereas between 16 and 30 weeks the ratio was lower. These data indicate that the increase in antichymotryptic activity in serum during pregnancy is greater than the antitryptic activity.

Both serum and amniotic fluid were found to contain small proportions of heat stable trypsin inhibitory activity. The heat stable fraction accounted for 3 to 5 per cent of the total activity in both control and pregnancy serum. On the other hand, in amniotic fluid the proportion varied from 8·7 to 15·6 per cent. The heat stable trypsin inhibitor was separated by chromatography on DEAE-cellulose both from serum and amniotic fluid. The heat labile inhibitors including α–1 antitrypsin and α–2 macroglobulin were eluted from the column at low ionic strength whereas the heat stable activity was separated by eluting with buffer of high ionic strength. A typical elution profile of amniotic fluid inhibitors is shown in Figure 5. A somewhat similar pattern was observed in serum, with low activity of the heat stable fraction. There was no correlation between the proportion of heat stable inhibitory activity to total activity in amniotic fluid with respect to gestational age. The results on eight samples after chromatography are shown in Table IV.

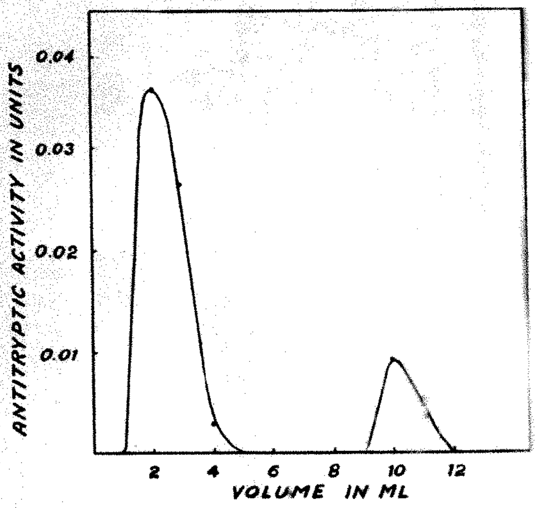

FIG. 5

Elution profile of amniotic fluid inhibitors on DEAE cellulose.

DISCUSSION

The present study on protease inhibitors in amniotic fluid confirms the findings of Sutcliffe and Brock (1973). It was observed that protease inhibitory activity in amniotic fluid increased from 12 to 20 weeks of gestation and thereafter remained in the same range up to 30 weeks. Our data on the range of individual variation (five-fold, taking the whole of the gestation period) agree well with the data of Sutcliffe and Brock (1973). We find that while amniotic fluid levels reached a peak value by 20 weeks gestation, maximal values were observed in serum around 30 weeks. Further, the fall in serum levels at term was not as great as in amniotic fluid. For antichymotryptic activity, it was observed that there was an early rise in serum compared to antitryptic activity. Moreover, the ratio of antitryptic to antichymotryptic activity was lower between 16 to 30 weeks compared to the values in early and late pregnancy or in the controls. In contrast, the ratio in amniotic fluid remained almost constant throughout. These data suggest that during pregnancy there is an increase in the serum of a factor with preferential action on chymotrypsin. This factor

TABLE IV

Heat stable trypsin inhibitor in amniotic fluid

No.	Gestation (weeks)	Total antitryptic activity (units/dl)	Heat stable inhibitor activity (units/dl)	Per cent of heat stable inhibitor activity
1	12	7·20	1·12	15·5
2	12	8·40	1·24	14·8
3	14	6·00	0·77	12·9
4	16	12·00	1·52	12·7
5	20	14·40	1·97	13·7
6	22	23·58	2·05	8·7
7	24	14·74	1·33	9·0
8	28	20·76	3·24	15·6

which could presumably be α–1 antichymotrypsin (Ohlsson and Akesson, 1976) does not appear to gain entry to amniotic fluid unlike α–1 antitrypsin. Further studies are needed to confirm this suggestion.

An interesting observation is the higher proportion of a heat stable trypsin inhibitor in amniotic fluid compared to serum in pregnancy. It has been reported that a heat stable trypsin inhibitor is found in larger amounts in urine during pregnancy (Faarvang, 1965). The concentration gradient of this factor between serum and amniotic fluid was found to be in the range of 1·8 to 2·5. The gradient for total antitryptic activity on the other hand, varied between 11·8 to 37·3 at different stages of pregnancy which agreed well with the reported values for α–1 antitrypsin (Sutcliffe and Brock, 1973). The low gradient for the heat stable inhibitor suggests that its origin in amniotic fluid may not be exclusively from maternal serum.

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SCANNING ELECTRON MICROSCOPIC APPEARANCES IN THE AMNION IN POLYHYDRAMNIOS AND OLIGOHYDRAMNIOS

BY

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Summary

The appearances of the amnion from two patients with polyhydramnios and two patients with oligohydramnios are described. In polyhydramnios the amnion showed a normal incidence of all the four patterns found in normal amnion at term and no abnormal features were noted. In oligohydramnios, two of four normal patterns predominated and intercellular canals were sparse.

THE scanning electron microscope is a relatively new instrument and, to our knowledge, there is no previous description of the surface ultrastructure of the amnion from patients with polyhydramnios and oligohydramnios. This study was undertaken to remedy this defect.

METHODS

Two patients with polyhydramnios and two with oligohydramnios who were delivered at the City Hospital, Nottingham, were studied. The diagnoses were based on clinical examination before delivery; amniotic fluid volume was not measured. In both patients with polyhydramnios the volume of amniotic fluid was grossly excessive. In one patient, the infant weighing 4080 g was delivered by elective Caesarean section and copious amniotic fluid was noted at operation. The second patient was admitted to hospital at term with clinical hydramnios which was confirmed at surgical induction of labour although the volume of fluid was not measured; the infant weighed 4580 g.

One of the patients with oligohydramnios was admitted at term because of weight loss and low serum levels of placental lactogen. At surgical induction, a small amount of blood stained amniotic fluid was obtained; the infant weighed 2840 g and had an Apgar score of 4 at five minutes. Active resuscitation was necessary and spontaneous respiration was not established for 11 minutes.

The second patient with oligohydramnios was admitted 12 days after the estimated date of delivery for induction of labour. No amniotic fluid was obtained and none drained during labour or delivery. The infant weighed 3270 g and had an Apgar score of 10 at five minutes.

The placentae were obtained immediately after delivery and the following samples were taken from macroscopically normal areas: one piece from the umbilical cord 3 cm above its insertion into the placenta; two pieces from the reflected amnion, one approximately 3 cm out from the placental margin and one near the point of rupture of the membranes; five further pieces from the reflected amnion around the circumference of the placenta near the placental margin.

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The pieces of amnion were gently stripped from the chorion and, to keep them flat, were mounted on a fine metal grid with their epithelial surface uppermost. Within 30 minutes of delivery, the selected pieces were placed in fixative (3 per cent glutaraldehyde in 0·12 M phosphate buffer at pH 7·4) for two hours. After fixation they were washed thoroughly in phosphate buffer and then dehydrated through 50, 70, and 90 per cent and absolute acetones. They were stored in absolute acetone at 4°C until the next stage.

The samples were dried in a Polaron E 3000 Critical Point Drying Apparatus using liquified carbon dioxide as the drying liquid. They were then mounted on a stub, coated with gold in a vacuum coating unit, and examined in a Cambridge Stereoscan 2A operating at 10 kV.

RESULTS

The samples of amnion showed a wide range of appearances at low magnification (approximately ×500); these corresponded with the basic patterns in normal amnion (Pollard *et al*, 1976) which are summarized below:

Pattern 1: A flat sheet of polygonal cells with definable cell boundaries. Intercellular canals are present but not numerous.

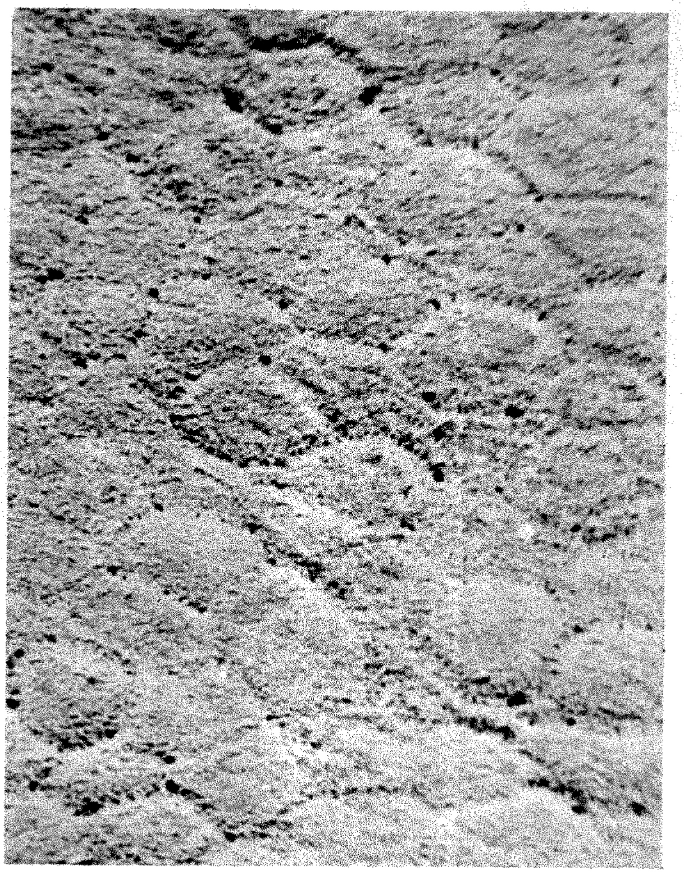

FIG. 1

Pattern 1 from a patient with polyhydramnios (×5300).

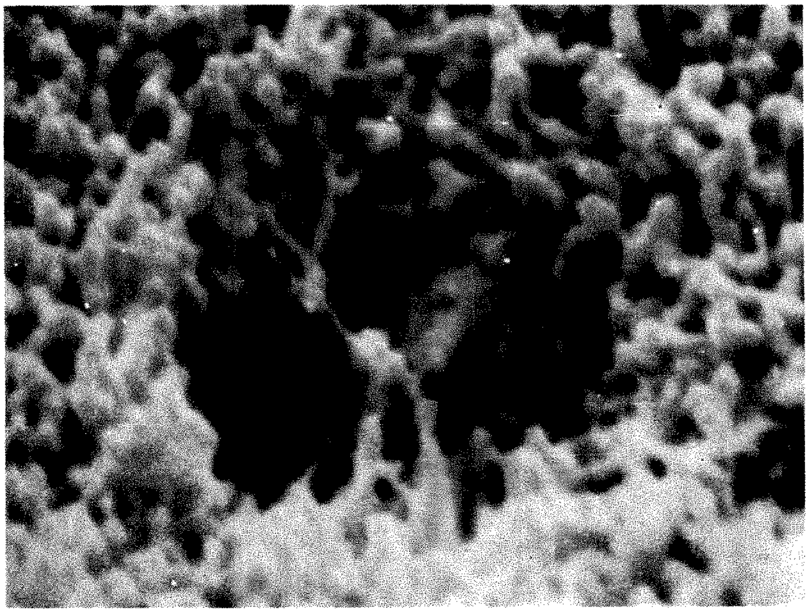

FIG. 2

An intercellular canal, with reticular strands in its depths, from a patient with polyhydramnios (× 45 000).

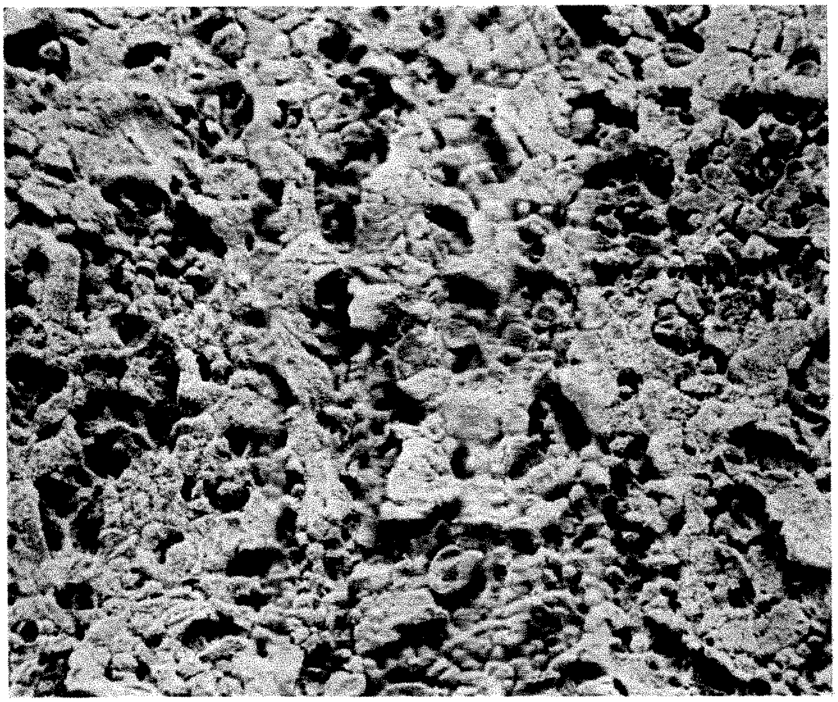

FIG. 3

Pattern 4 from a patient with oligohydramnios (× 2000).

Pattern 2: A sheet of polygonal cells with an irregular scattering of 'spider cells'—slightly larger, usually oval cells surrounded by an intercellular space traversed by thin strands of cytoplasma. Intercellular canals are more numerous than in pattern 1.

Pattern 3: A sheet of somewhat elongated polygonal cells, tending to lie in parallel rows, with an irregular scattering of larger, flatter cells. Intercellular canals are very sparse.

Pattern 4: An irregular sheet of rounded cells with deep clefts between adjacent cells. There is more cohesion between cells in some areas, giving a polygonal surface pattern.

In samples from the patients with polyhydramnios, all four basic patterns were observed. Patterns 1 (Fig. 1) and 2 were commonly found; pattern 3 was the least common. This was also the case in our normal series. At higher magnifications, microvilli were observed on the cells of all the basic patterns. In some samples they were plump and in others spindly; this variation in morphology was found in normal amnion. The intercellular canals (Fig. 2) were also of similar appearance and dimensions to those in normal amnion and appeared to be present in normal numbers, although comparative counts were not feasible.

In samples from the patients with oligohydramnios, patterns 1 and 4 (Fig. 3) were by far the most common; pattern 2 was found only in two samples from one of the patients and pattern 3 was not observed. Microvilli (Fig. 4) were present on most cells and showed morphological variability similar to that in normal amnion. In a few samples showing pattern 4, small groups of cells were denuded of microvilli.

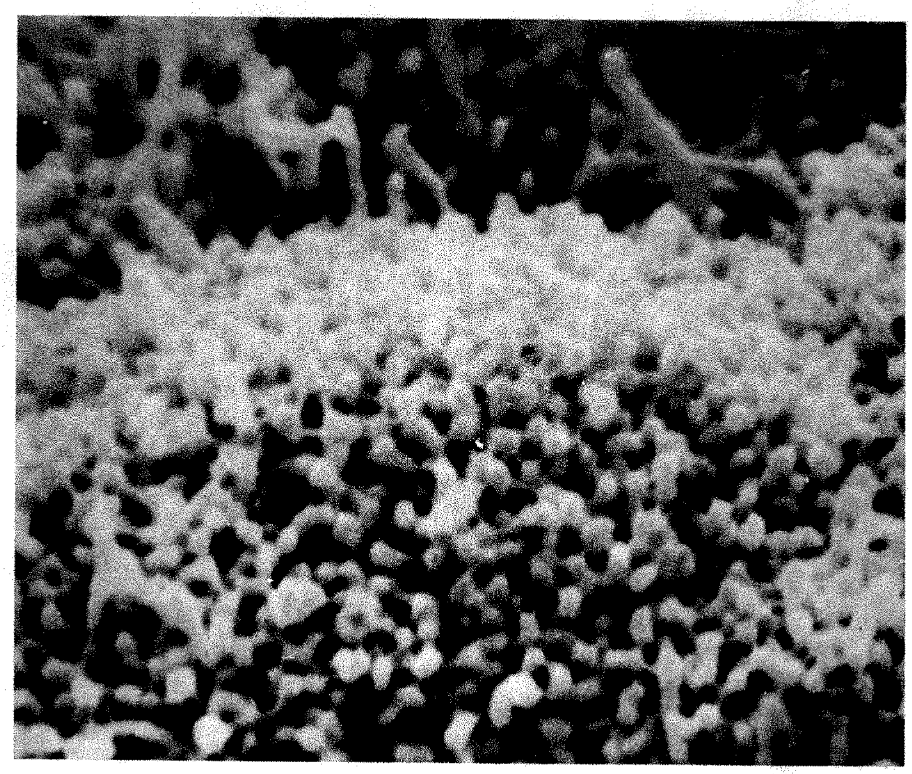

FIG. 4

Microvilli from a patient with oligohydramnios; the low power view showed pattern 4 (×40 000).

Intercellular canals in pattern 1 were very sparse.

Umbilical cord samples from all four patients showed normal morphology, with sheets of polygonal cells fitting closely together and no visible intercellular channels. Desquamation of the surface sheets revealed similar cells beneath. The cells were densely covered with microvilli, varying in morphology as in the amnion. Fusion of microvilli to form short ridges was seen in one of the patients with polyhydramnios. We had also observed these ridges in umbilical cord samples from normal placentas.

A surface coating of interlacing strands or clumps of material was present in a few samples, but was not more common than in our normal series.

DISCUSSION

The limitations imposed by our method, and the problems of sampling the amnion adequately in view of the variable patterns observed, were discussed in our previous paper (Pollard *et al*, 1976). Nevertheless, our two patients with polyhydramnios provided examples of all the four basic patterns we had observed in the normal amnion, in similar frequencies to those we found in the normal series. The microvilli showed the same variations as the normal samples. Intercellular canals also appeared normal in number and dimensions. We therefore found no evidence of abnormal surface morphology of the amnion in polyhydramnios.

Many patients with polyhydramnios have a higher than normal concentration of sodium in the amniotic fluid (Seeds, 1968). *In vitro* incubation of amnion in various preservative solutions (Hoyes, 1972) indicated that marked changes in the fine structure of the amniotic epithelium could occur when the concentration of ions such as sodium were higher than normal. It is of interest, therefore, that in our two cases of polyhydramnios the amnion showed normal surface morphology.

In the patients with oligohydramnios, pattern 4 (Fig. 3) suggests that the cell sheet is breaking up, possibly by shrinkage of the cells. This could be an osmotic effect linked to the paucity of amniotic fluid.

The scarcity of intercellular canals in pattern 1 in our cases of oligohydramnios may also be related to the low amniotic fluid volume. Hoyes (1975) considered that the efficiency of reabsorption of amniotic fluid was probably dependent on the diameter of the intercellular spaces. The closure of the canals in oligohydramnios could be a means of limiting the fluid exchange through the amnion in this condition, but examination of further samples will be necessary to confirm that this is a constant feature of oligohydramnios.

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We thank the staff of the Labour Suite at the City Hospital, Nottingham, for their co-operation, Dr Nehru, Mr L. Green and Mrs M. Villiers at the Wolfson Institute of Interfacial Technology for their technical assistance with the scanning electron microscopy, and Mr W. Brackenbury at the University Department of Pathology, Nottingham, for the illustrations. We are also grateful for the use of the post-graduate library facilities at North and South Tees, where one of us (S. M. Pollard) is now residing.

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THROMBOPLASTIC AND FIBRINOLYTIC ACTIVITY OF HYDATIDIFORM MOLAR TISSUE AND VESICULAR FLUID

BY

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Summary

We found similar levels of thromboplastic activity in molar tissue, vesicular fluid and normal placental tissue from pregnancies matched for gestational age. The fibrinolytic activity of molar tissue and vesicular fluid was, however, increased and vaginal haemorrhage tended to be heavier when fibrinolytic activity was high ($p = 0 \cdot 06$). Subsequent choriocarcinoma occurred more frequently in patients with molar tissue which had low fibrinolytic activity ($p = 0 \cdot 08$).

THE incidence of malignant change in a hydatidiform mole which is 13·5 per cent in Singapore (Tow, 1964) has been said to range from 8·3 per cent (Brews, 1939) to 21·5 per cent (Chun *et al*, 1964). There is still no method of predicting which hydatidiform mole will become malignant.

Normal trophoblast, and presumably molar tissue, can be deported to distant organs via the blood stream (Schmorl, 1893; Jäämeri *et al*, 1965) and there are reports of maternal deaths due to massive pulmonary embolism of trophoblastic cells (Lippi *et al*, 1962). Deportation of trophoblastic tissue may cause varying degrees of intravascular coagulation and fibrinolysis (Tsakok, 1976). The growth and spread of any tumour is undoubtedly influenced by many different mechanisms, and extravascular fibrin deposition could favour malignant infiltration. O'Meara (1958 and 1968) found high thromboplastic activity in human tissue. Fibrinolytic activity was found to be low in carcinomas of epithelial origin and high in sarcoma (Cliffton and Grossi, 1955).

Tumour growth and metastasis could thus be related to the thromboplastic and fibrinolytic properties of tumours (Peterson, 1968).

We therefore investigated the thromboplastic and fibrinolytic activities of hydatidiform molar tissue and vesicular fluid, and also whether or not these measurements could be used to predict the occurrence of malignant change.

METHODS

Preparation of tissue

Hydatidiform molar tissue and placental tissue matched for gestational age were collected. The tissue was either tested immediately or stored for not more than six hours at −20°C.

Preparation of vesicular fluid

Larger fresh vesicles were washed in normal saline. A clean puncture was made and vesicular fluid was aspirated into a plastic syringe. The fluid was collected in plastic tubes and tested immediately or stored for not more than six hours at −20°C.

Measurement of thromboplastic activity

Tissue from eight hydatidiform moles and placentae from induced abortions matched for gestation were studied. From each tissue, 200 mg net weight was homogenized in 2 ml of Owren's buffer solution in a glass homogenizer at 1600 rpm for five minutes at 0°C. The homogenate was frozen to −20°C for at least 24 hours, then thawed and rehomogenized to complete cell destruction.

Thromboplastic activity was determined by measuring the clotting time of plasma in a recalcification system. The plasma used was obtained by collecting nine volumes of blood to one volume of 3·8 per cent Hepes citrate solution and centrifuging at 2000 *g* for five minutes. Plasma obtained from 30 normal donors was pooled.

A 0·2 ml volume of tissue homogenate in serial dilutions with Owren's buffer was mixed with 0·2 ml of pooled citrated plasma in the clotting tubes and incubated in a waterbath at 37°C. A 0·2 ml volume of 0·05 M calcium chloride solution was added and the time needed for formation of a fibrin clot was registered. A control clotting time was obtained by replacing the diluted tissue homogenate with the same volume of buffer. To measure the thromboplastic activity of molar vesicular fluid, 0·2 ml of fluid and its serial dilutions in Owren's buffer were used instead of the tissue homogenate.

Measurement of fibrinolytic activity

The fibrinolytic activity of molar tissue was studied by the fibrin plate method (19 patients) as described by Nilsson and Olow (1962) and by the fibrin slide method (8 patients) of Todd as modified by Pandolfi (1972).

Fibrin plate method

Human fibrinogen (lyophilized) was reconstituted with phosphate buffer to a final working strength of 250 mg/100 ml. The fibrinogen solution was clotted with five units of thrombin in a Petri dish and allowed to stand on a flat surface for 30 minutes before use. Tissue fragments measuring approximately 3 mm × 3 mm × 3 mm were placed on the plates and incubated at 37°C for 18 hours. The area of lysis was read on graph paper.

Fibrin slide method

Tissue frozen to −40°C was cut in a cryostat into sections of 8 μm thickness, and each section was covered with a fibrin film which was 0·07 mm thick. The sections were incubated for 0, 30, 60 or 90 minutes in a moist chamber at 37°C before fixation in formalin and staining by Giemsa and Harris haematoxylin. Fibrinolysis was reflected by the clear lytic areas, and each slide was given a score: 0·5 for punctate areas of lysis in some sections, 1 for punctate areas of lysis in all sections, 1·5 for large areas of lysis in all sections, 2 for confluent zones of lysis in some sections, 2·5 for confluent zones of lysis in all sections and 3 for a zone of lysis surrounding the section.

Measurement of tyrosine content

The tyrosine content of the tissue homogenate and vesicular fluid were measured by the method of Lowry *et al* (1951). A 0·2 ml sample of tissue homogenate was boiled in 1 ml of 10 per cent sodium hydroxide for half an hour; 3 ml of 20 per cent sodium carbonate were added followed by distilled water to make up 10 ml; 1 ml of Folin Ciocalteu's reagent was added and the colour reaction was allowed to develop for 30 minutes. The intensity of the colour developed was measured by spectrophotometery at 650 nm. To determine the tyrosine content of the tissue homogenate and vesicular fluid, the reading obtained was compared with those obtained from tyrosine standards.

RESULTS

Thromboplastic activity

The clotting times of recalcified standard plasma with increasing dilutions of tissue homogenate and vesicular fluid are shown in Figure 1. Molar tissue had as much thromboplastic activity as placental tissue. Vesicular fluid, however, had lower thromboplastic activity than both.

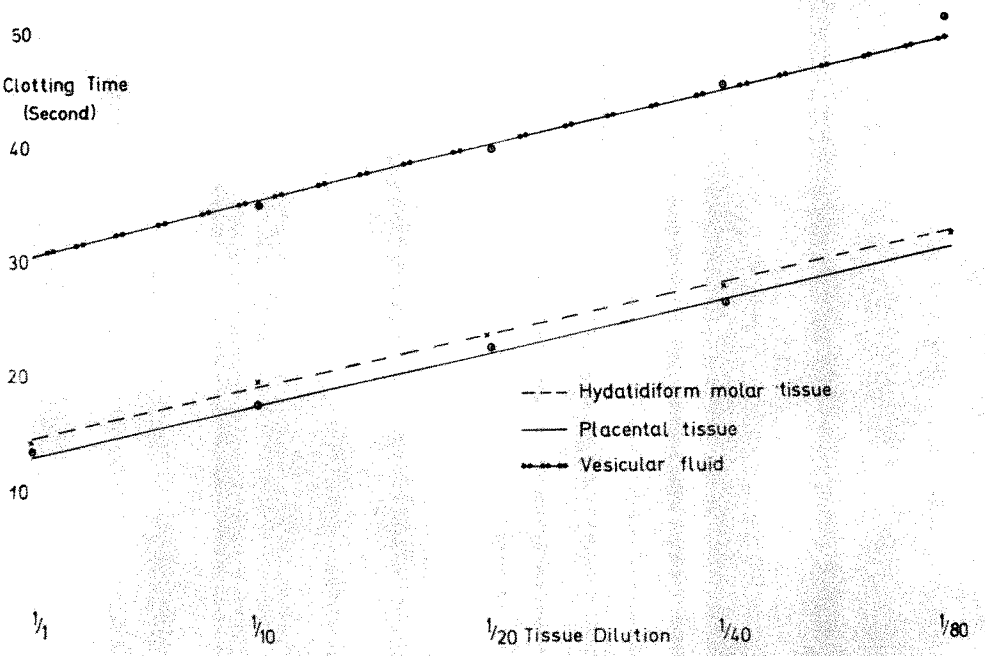

FIG. 1

Clotting time with increasing tissue dilution or fluid dilution.

Fibrinolytic activity (fibrin plate method)

The areas of lysis produced by molar tissue, placental tissue and vesicular fluid are shown in Figure 2.

In 14 out of 19 samples of molar tissue and in 9 out of 19 samples of vesicular fluid, the area of lysis was above 700 mm² whereas none of the 19 samples of placental tissue produced an area of lysis over 700 mm² and 10 of these samples showed no lysis at all.

Figure 2 also gives information about vaginal bleeding and the subsequent development of choriocarcinoma.

The amount of bleeding was not recorded in 3 patients. In the 12 patients with molar tissue which produced more than 700 mm² and an existent record of the amount of bleeding, 10 had moderate to heavy vaginal bleeding. Of the 4 patients with molar tissue producing less than 700 mm² of fibrinolysis and a known amount of vaginal bleeding, only one had a moderate loss. Vaginal bleeding tended to be less in those patients with molar tissue of lower fibrinolytic activity (Fig. 2; Fisher's exact test, $p = 0{\cdot}06$).

Two out of 14 patients with molar tissue of high fibrinolytic activity (more than 700 mm² of lysis) subsequently developed choriocarcinoma as against 3 out of 5 with low fibrinolytic activity (less than 700 mm² of lysis). ($p = 0{\cdot}08$; Fisher's exact test).

Fibrinolytic activity (fibrin slide method)

The lysis scores for eight hydatidiform moles and eight placentae matched for gestational age are shown in Figure 3. Six placental samples as against only one sample of molar tissue produced no lysis.

Tyrosine content

The tyrosine content (mean ± SD) for placental tissue, molar tissue and vesicular fluid was, respectively, 0·95 ± 0·06 mg/100 mg, 0·72 ± 0·03 mg/100 mg and 1·48 ± 0·02 mg/100 mg.

DISCUSSION

Intravascular coagulation with widespread fibrin deposition has been found in patients who come to autopsy after aborting a hydatidiform mole. Recent studies have also shown some intravascular coagulation and secondary fibrino-

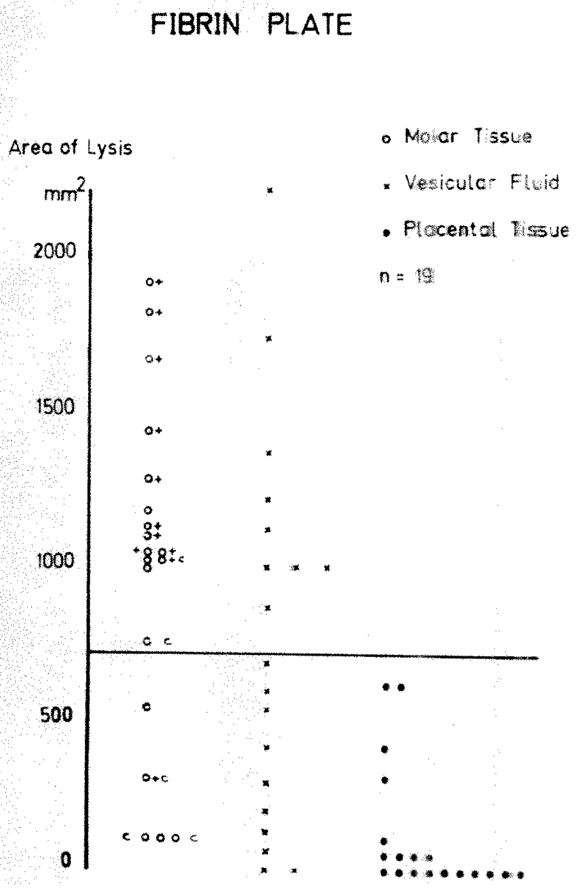

FIG. 2

Area of fibrinolysis produced by molar tissue ○, vesicular fluid × and placental tissue●, using fibrin plate technique. An arbitrary dividing line has been drawn at 700 mm². Heavy vaginal bleeding (+) and the subsequent development of choriocarcinoma (c) are shown alongside the results for molar tissue.

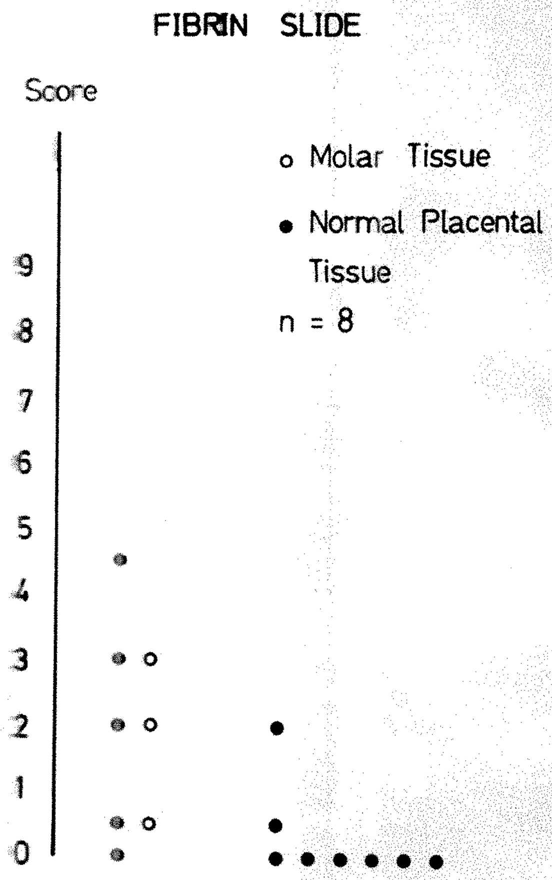

FIG. 3

Fibrinolysis score (Slide method) for molar tissue (○) and normal placental tissue (●).

lysis in molar pregnancies with no bleeding and no clinical signs of coagulopathy (Tsakok, 1976). Our study has shown that, like placental tissue, molar tissue and vesicular fluid have thromboplastic activity and so the entry of either into the maternal circulation could cause intravascular coagulation. The thromboplastic effect of molar tissue or molar fluid does not seem to be directly related to the amount of tyrosine-containing protein it contains.

It was interesting to find that both molar tissue and vesicular fluid had fibrinolytic activity whereas normal trophoblastic tissue of comparable gestational age did not.

The tendency for the amount of vaginal bleeding to be related to fibrinolytic activity of molar tissue suggests that this activity may play a role in determining the pattern and amount of bleeding from the uterus.

Although levels of statistical significance are not reached, our results also suggest that molar tissue of low fibrinolytic activity has a greater potential for malignancy.

It could be that fibrinolytic activity is only increased in tissue that is detached from the uterus; the normal placenta and malignant trophoblastic tissue, both having low fibrinolytic activity, being more adherent to the uterus. This possibility requires further investigation. If it can be shown that decreased fibrinolytic activity of molar tissue is associated with higher risk of subsequent malignant change, one would have a method of selecting patients for very close follow-up.

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ACUTE MENOPAUSAL TRANSITION ASSOCIATED WITH CLOMIPHENE THERAPY: TWO CASE REPORTS

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Summary

Two patients presenting with anovulation and secondary infertility were treated with clomiphene citrate. Intermittent blood samples were obtained for the first three months of therapy in each case. One patient failed either to ovulate or menstruate in response to clomiphene and the other patient had only two episodes of cyclical bleeding before she too became amenorrhoeic. Hormonal analyses revealed that both women had undergone a rapid and precocious menopausal transition which has persisted throughout the subsequent three years.

SINCE clomiphene citrate was first described in 1960 for ovulation induction (Kistner and Smith, 1960) it has been used extensively for the treatment of patients with all types of infertility although possibly its greatest potential is in the treatment of secondary amenorrhoea or oligomenorrhoea (Garcia *et al*, 1977). Unfortunately not all these patients respond successfully by ovulating (Poliak *et al*, 1973) and conceiving for reasons which are largely unknown.

The present report describes two patients from a consecutive series of 20 women who were carefully monitored following ovulation induction with clomiphene. Treatment in these two patients appeared to precipitate a precocious or premature menopause (ovarian failure, WHO Consultative Document, 1976).

METHODS

Blood samples were obtained daily or every other day throughout the first three courses of clomiphene treatment. Plasma was separated within 60 minutes and stored at −20°C until assayed for LH, FSH, oestradiol and progesterone. Plasma concentrations of prolactin (PRL), androstenedione and testosterone, in addition to the above hormones, were estimated in one pre-treatment sample from each patient. All the hormones were measured by radioimmunoassays described elsewhere (Lenton *et al*, 1978). Antibodies were kindly provided by the National Pituitary Agency (anti-LH, anti-PRL), Dr W. Butt (anti-FSH), Dr R. Scaramuzzi (anti-oestradiol) or were obtained commercially. Results were expressed as U/l of the reference preparation 2nd IRP-HMG (LH and FSH) and mU/l of the research standard 71/222 (PRL), both supplied by the National Institute for Biological Standards and Control, London.

CLINICAL HISTORIES

Patient 1

The patient, aged 29, presented with oligomenorrhoea and infertility of three years duration following the birth of her first child. Routine investigations (including hysterosalpingography, thyroid function tests and her husband's semen analysis) were all within normal limits. Vaginal cytology showed little evidence of oestrogenic activity (as assessed by the karyopyknotic index) and no endometrium was obtained at curettage. The haemoglobin was 13·3 g/dl and 17 oxo- and oxogenic steroids were normal at 7·4 mg/24 hours and 15·4 mg/24 hours respectively. Pretreatment hormone levels were as follows: LH 40·5 U/l; FSH 73·0 U/l; PRL 275 mU/l; oestradiol 194 pmol/l; androstenedione 3·3 nmol/l and testosterone 3·9 nmol/l. Despite the already elevated gonadotrophin concentrations, ovulation induction was attempted. Clomiphene (50 mg daily) was given for five days and when

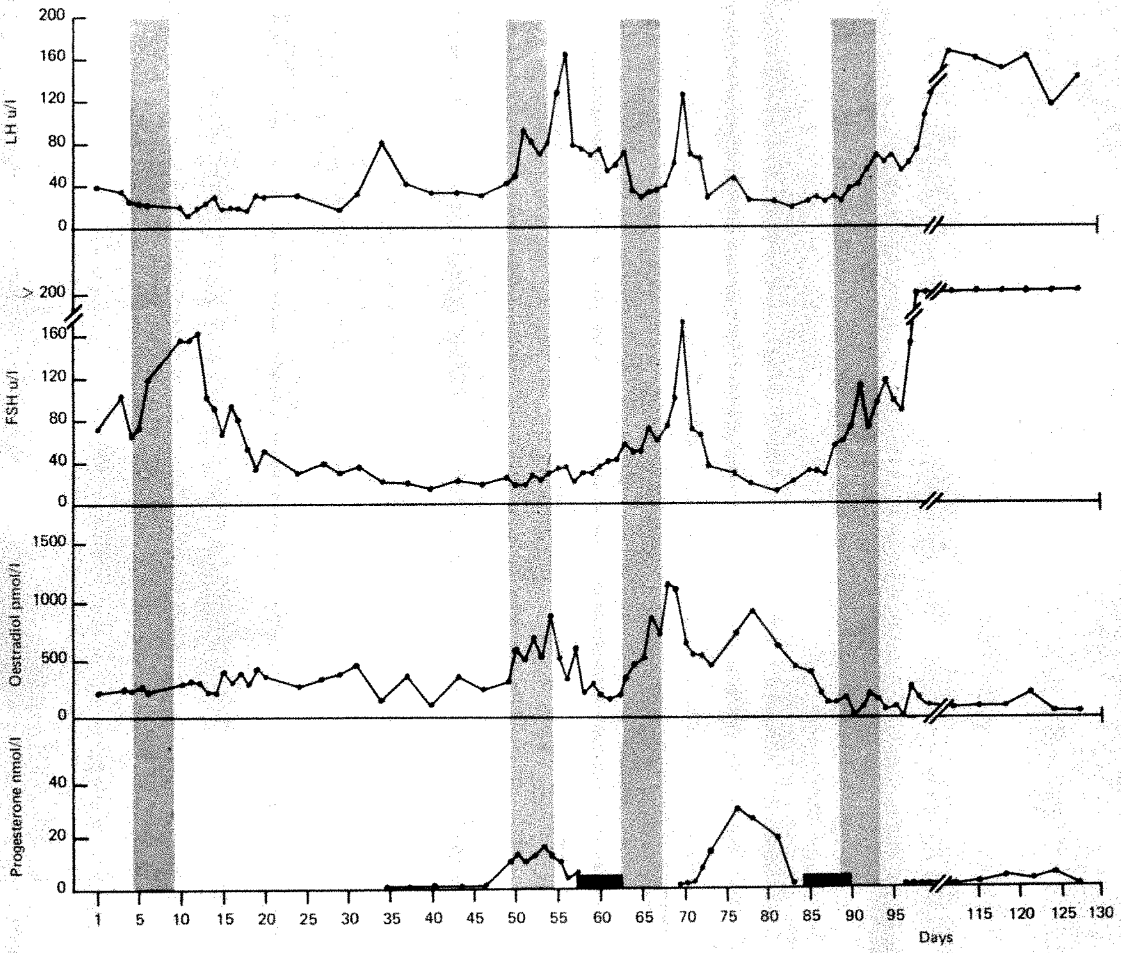

FIG. 1

The plasma concentrations of LH, FSH, oestradiol and progesterone during treatment of Patient 1. The shaded bars show the duration and timing of clomiphene administration and the filled block indicates menstruation.

there was no apparent response (flat basal temperature recording and no period), a second course (100 mg daily for five days) was commenced 49 days after the first. There followed an apparently normal menstrual period after an interval of only eight days (Fig. 1) which hormonal analysis subsequently showed was because the second course of clomiphene had inadvertently been given during a preceding luteal-like phase. A third course of clomiphene (100 mg daily) was accordingly given from Day 5 to 9 of this cycle and another episode of bleeding occurred after an apparently normal cycle lasting 27 days. However the next and all subsequent courses of treatment at the same dose level were without further clinical effect and there has been no spontaneous resumption of menses in the subsequent three years.

Patient 2

The patient, a 37-year-old Indian lady presented with a history of irregular cycles and secondary infertility of 18 months duration. She had had one normal pregnancy nine years previously. Again, routine investigations were within normal limits and only a small amount of non-secretory slightly hyperplastic endometrium was obtained at curettage. Pre-treatment hormone concentrations were as follows: LH 14·0 U/l; FSH 10·5 U/l; PRL 450 mU/l; oestradiol 445 pmol/l; androstenedione 0·43 nmol/l; testosterone 0·8 nmol/l and progesterone 1·78

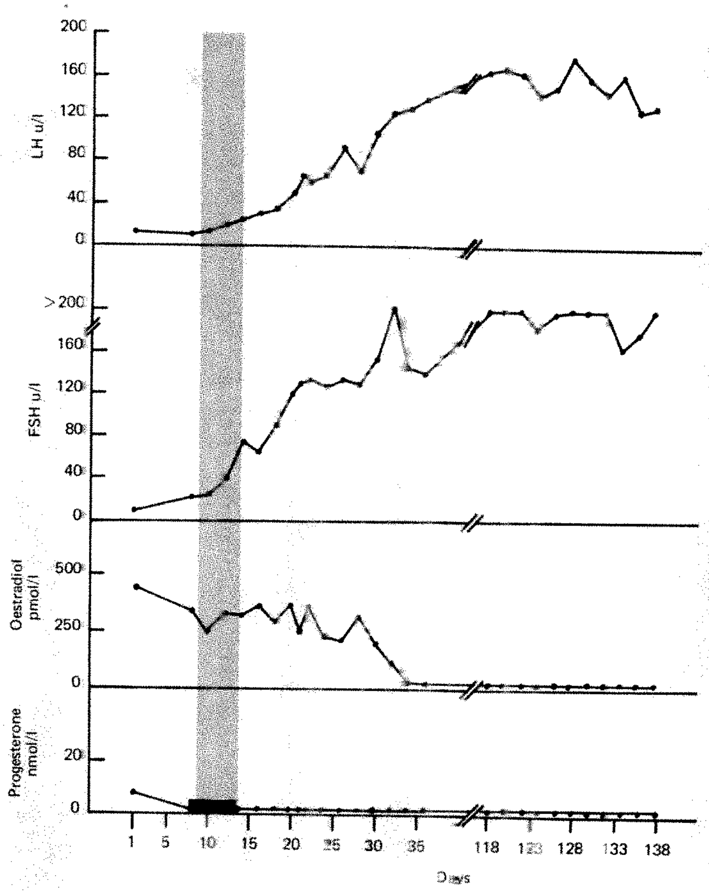

FIG. 2

Plasma concentrations of LH, FSH, oestradiol and progesterone during treatment of Patient 2. The shaded bar represents the timing of clomiphene administration and the filled block indicates menstruation.

nmol/l. The patient started menstruating spontaneously the day before clomiphene treatment (at 50 mg daily for five days) began. Her previous two cycles had been of 27 and 25 days duration and the above pre-treatment blood sample corresponded to Day 18 of a 25 day cycle (Fig. 2). Both the hormone concentrations and basal temperature recording suggested that these cycles were anovulatory. There was no clinical response to clomiphene, the basal temperature recording was consistent with anovulation and there were no further episodes of bleeding. Amenorrhoea has persisted for the subsequent three years.

ENDOCRINE RESULTS AND INTERPRETATION

Patient 1

Patient 1 had not menstruated for several months before treatment was initiated and the finding of slightly elevated peripheral concentrations of both LH and FSH suggested that some degree of ovarian failure was already present. The first course of clomiphene was associated with an exaggerated response by FSH but not by LH. There was however, no increase in oestradiol secretion and no evidence of any follicular development (Fig. 1). However, after about 45 days some luteal-like activity was observed with simultaneous elevation of both oestradiol and progesterone concentrations. This phase lasted about 10 days and was followed by an episode of bleeding. There did not appear to be an LH surge associated with the start of the luteal phase, although LH levels subsequently rose and parallelled the changes seen in oestradiol and progesterone concentrations. Unfortunately a second course of clomiphene was given at this time which may have been responsible for the elevation in LH and the dissociation between LH and FSH concentrations. The third course of clomiphene in this patient produced smaller alterations in both LH and FSH but stimulated apparently normal follicular development with oestradiol secretion. This follicular phase was followed by a probable ovulatory surge of both LH and FSH (although the latter was greatly exaggerated), and a luteal phase lasting 14 days. The basal temperature recording also showed normal appearances. The fourth course of clomiphene produced marked increases in both LH and FSH but there was no concomitant ovarian response and gonadotrophin concentrations continued to rise until within a week they had reached menopausal levels. Steroid secretion by the ovary was, and has remained, low.

Patient 2

Patient 2 had experienced irregular cycles for some months before treatment. Her basal temperature recording showed these to be anovulatory cycles separated by episodes of heavy bleeding. Her final period occurred one day before the first course of clomiphene (Fig. 2). Pre-treatment gonadotrophin levels were within the normal range and gave no suggestion that this woman was peri-menopausal. However, following 50 mg of clomiphene, gonadotrophin concentrations rose rapidly and after 10 days oestradiol secretion decreased abruptly. By this time the gonadotrophins were within the menopausal range where they have remained since.

DISCUSSION

The hormonal changes occurring during the transition from the peri-menopausal to the menopausal state have not been extensively documented and we could find only two other examples of what was probably the final menstrual cycle in a woman's reproductive life (Sherman *et al*, 1976; Van Look *et al*, 1977). These authors have, however, analysed a number of cycles from peri-menopausal women and the profiles they have obtained are similar to the hormone patterns initially observed in Patient 1 (Sherman and Korenman, 1975). It would appear that most women experience cycles with bizarre hormonal relationships during the peri-menopausal period (Adamopoulos *et al*, 1971), which may last several years (Keettel and Bradbury, 1964). Our two patients apparently did not have long peri-menopausal phases and it seems likely that clomiphene, by stimulating ovarian function (via increased gonadotrophin secretion) may have precipitated ovarian failure and thus the onset of true menopause.

The dissociation between the secretion of LH and FSH is very obvious in the first cycle of

Patient 1 and supports the hypothesis put forward by Sherman and Korenman (1975) that there may be an ovarian regulatory hormone analogous to the 'inhibin' thought to exist in men. This factor would reduce progressively with age and would be important in the regulation of FSH secretion. It is possible that clomiphene may accelerate the age-related reduction in 'inhibin'.

The transition to the menopausal state occurred very rapidly in both our patients with gonadotrophin levels rising simultaneously to the menopausal range over the space of a few days. Final concentrations of FSH were about twice the concentration of LH and the whole pattern was very similar to the acute onset menopause following oophorectomy (Monroe *et al*, 1972). Despite the abruptness of the transition, neither of these two women experienced any menopausal symptoms. It is doubtful whether, once the true menopausal state has been reached, ovarian function is restorable (Keettel and Bradbury, 1964) although it is possible to see from our data how a woman on the verge of the menopause may still have occasional apparently normal cycles (Patient 1) and could possibly conceive. Clinically these patients would have been classed as treatment failures although the actual cause of this failure would not have been apparent without a certain amount of endocrine information. Early recognition of the menopausal state prevents undue prolongation of treatment and unnecessary distress to the patient.

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AN EXTRAPERITONEAL OPERATION FOR VAGINAL VAULT SUSPENSION TO THE ANTERIOR ABDOMINAL WALL

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Summary

A completely extraperitoneal abdomino-vaginal operation for vaginal inversion, using Mersilene mesh, is described. There were no major postoperative complications and at follow-up of the 13 patients at 10 to 26 months after operation the results were satisfactory.

THE difficulty of curing vaginal vault prolapse is evidenced by the large number of operative procedures proposed over the past 40 years (see list of references). I describe a new operation which is aimed at correcting the inversion and allowing normal coitus. Details of the 13 patients treated are shown in Table I. In 12 patients the apex of the vaginal vault was outside the vulva and in one it was at the introitus.

OPERATIVE TECHNIQUE

The patient was placed in a modified lithotomy position allowing access to both abdomen and vagina.

Through a transverse incision, 5 cm above the upper border of symphysis pubis, the external oblique aponeurosis was opened at the lateral edge of the rectus muscle on one side. Using the forefinger, a tunnel was made down the side wall of the pubis until the medial border of the obturator foramen was felt. A similar tunnel was fashioned on the opposite side.

A No. 16 Foley catheter was inserted into the bladder, and the bladder was emptied but the catheter was retained so that its balloon served as a landmark. The apex of the vagina was grasped by Allis forceps at the 3 and 9 o'clock positions. The transverse scar of the previous operative closure was usually situated anterior to these points.

A transverse incision was made between the Allis forceps and the vaginal epithelium was separated from the underlying tissue in all directions for 3 to 4 cm. If an enterocele was present, the dissection was extended to the enterocele sac. The blunt dissection was extended laterally on both sides until the inner surface of the pubic ramus was felt. With one finger passed abdominally into the tunnel, the operator passed a curved uterine dressing forceps into the vaginal tunnel on the same side, until the tip of the forceps could be felt by the abdominal finger. With the forceps and finger tip in constant contact, the triangular ligament was perforated by gentle pressure, and the perforation was enlarged by slightly opening the forceps.

The forceps was then passed upwards through the abdominal tunnel until the tip of the forceps could be seen in the abdominal incision. Then the end of a No. 1 chromic catgut suture was placed in the mouth of the forceps and pulled down through the tunnel and out through the vaginal incision, forming a pilot suture. The procedure was repeated on the opposite side (Fig. 1).

Now the Mersilene mesh, previously shaped as in Figure 2 and sterilized, was placed in front of the vaginal incision. The wider middle part of the mesh was fixed to the vaginal vault by 6 Mersilene 00 sutures. Either end of the Mersilene

TABLE I

Details of patients with vaginal vault prolapse

No.	Age	Parity	Prior surgery	Additional complaints	Interval* (months)	Postoperative hospital stay (days)
1	60	7	C/S, Subtotal hysterectomy	None	24	10
2	40	6	T.A.H.	None	1	8
3	35	5	C/S T.A.H.	Stress incontinence	9	8
4	40	4	T.A.H.	None	48	8
5	65	12	T.A.H.	None	36	10
6	35	8	T.A.H.	None	6	12
7	50	5	Vag. hyst.	Stress incontinence	6	7
8	35	8	Vag. hyst.	None	3	7
9	45	5	Vag. hyst.	None	1	10
10	40	4	Vag. hyst.	None	5	8
11	40	10	Vag. hyst.	None	4	7
12	35	5	T.A.H.	None	3	8
13	39	4	Vag. hyst.	None	7	9

C/S = Caesarean section; T.A.H. = Total abdominal hysterectomy; Vag. hyst. = Vaginal hysterectomy.
* Interval between primary operation and occurrence of vault prolapse

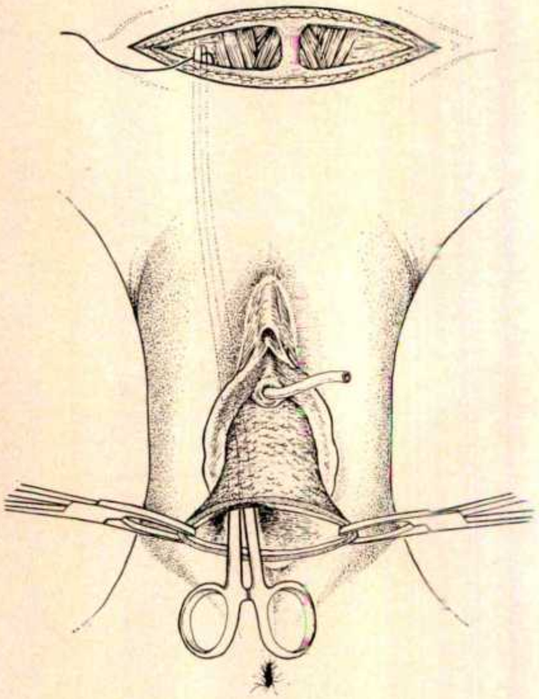

FIG. 1

A uterine dressing forceps is passed through the vagino-abdominal tunnel and a pilot suture is passed through the tunnel by the tip of the forceps.

mesh was then tied to the pilot sutures on each side and the abdominal end of the pilot sutures were gently pulled up until the ends of the Mersilene mesh emerged into the abdominal incision (Figs. 3 and 4).

About 50 ml of sterile 0·1 per cent methylene blue was instilled into the bladder through the Foley catheter to exclude any penetration of the bladder during the course of the operation. No such complication occurred in my series.

The vaginal incision was closed with interrupted mattress sutures of catgut No. 0.

Both abdominal ends of the Mersilene mesh were then pulled upwards, so that the vagina stood in its normal position without excessive tension. The end of the Mersilene mesh was secured to the aponeurosis of the abdominal wall by Mersilene 00 sutures, and the abdominal wall was closed.

The vagina was packed gently and the Foley catheter was left in place for 24 hours but no antibiotics were given.

RESULTS

The 13 patients were followed from 10 to 26 months. In all, the vaginal vault remained well supported, with excellent vaginal length and capacity.

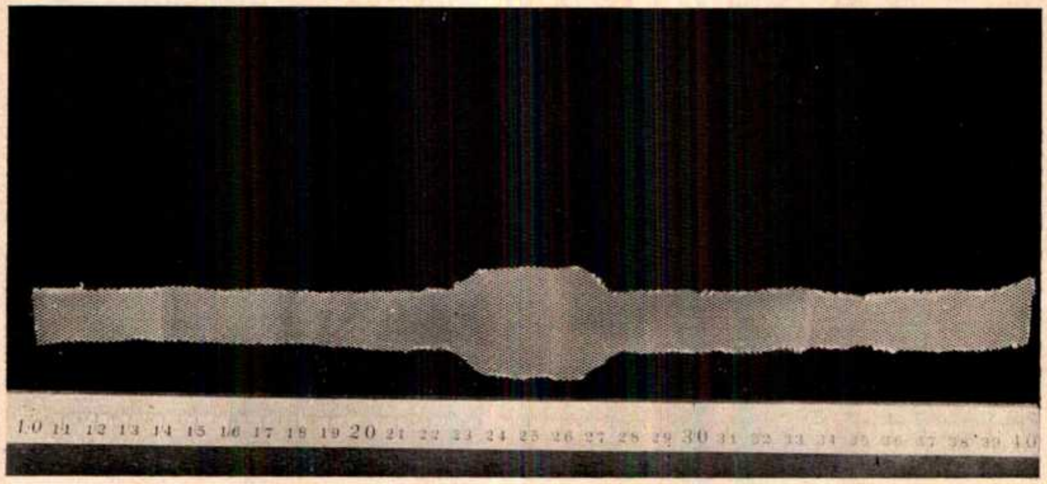

FIG. 2

Mersilene mesh shaped and ready for use.

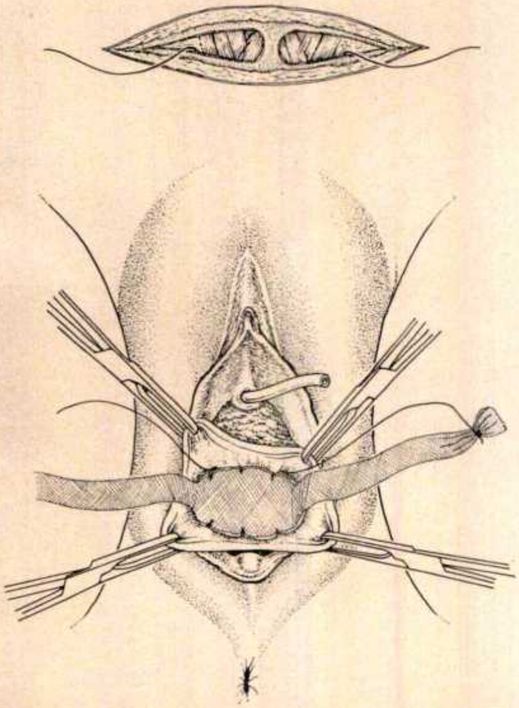

FIG. 3

Fixation of Mersilene mesh to the vaginal vault. The pilot sutures are tied to the either ends of Mersilene mesh.

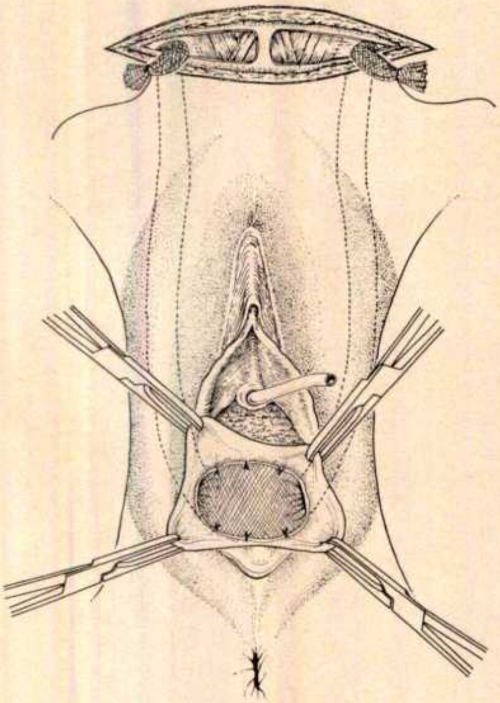

FIG. 4

Mersilene mesh is now in place and either ends are passed through the tunnels and out from the abdominal incision by pulling-up the pilot sutures.

In one woman the operation was combined with removal of the cervical stump, and in two patients a Marshall-Marchetti-Krantz operation was also performed because of stress incontinence. In both cases urinary incontinence was controlled for the duration of follow-up which was 7 months and 1 year respectively.

In the one patient with an enterocele, the problem was dealt with by extension of the dissection of the posterior lip of the vaginal incision to the enterocele sac. The sac was opened, dissected as high as possible and then closed with a highly placed purse-string No. 00 chronic catgut suture and the redundant peritoneum excised.

None of the 11 patients who were sexually active experienced dyspareunia. No postoperative infection developed and no granulation tissue or sinuses formed at the site of the vaginal incision.

All patients were pleased with the result of surgery and there were no recurrences of prolapse during the follow-up period.

COMMENT

The relatively high incidence of vaginal vault prolapse in Iranians may be attributed to such factors as inadequate diet, lack of maternity care in rural areas, and high parity.

The technique described is a simple modification of many operative procedures previously described for vaginal vault prolapse. The results so far have been promising and without serious complications.

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ANTENATAL SEARCH FOR FETAL β-THALASSAEMIA IN A TWIN PREGNANCY

BY

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Summary

The antenatal search for fetal β-thalassaemia in a twin pregnancy is described. Thalassaemia major was correctly excluded in both fetuses, and some of the problems of identifying patients at risk are outlined as well as those of obtaining fetal blood in the second trimester of pregnancy.

THE development of biochemical techniques and the increasing safety of antenatal fetal blood sampling now offer the prospect of antenatal diagnosis of β-thalassaemia major to couples at risk (Kan *et al*, 1977; Alter *et al*, 1976; Fairweather *et al*, 1978). This report describes the search for fetal β-thalassaemia in a twin pregnancy.

CASE REPORT

A 20-year-old Greek Cypriot was seen at 8 weeks gestation in her third pregnancy. She had one normal girl and her second pregnancy, which occurred while taking oral contraception, was terminated. At that time, at another hospital, the patient was found to have β-thalassaemia trait, as evidenced by microcytosis and a raised level of HbA_2 (Weatherall and Clegg, 1972).

Because of domestic problems, the patient initially sought termination of her third pregnancy, but subsequently changed her mind despite the fact that her husband, who intended to leave her, also had the β-thalassaemia trait. The patient was advised that there was a 1 in 4 chance of giving birth to a child with β-thalassaemia major, and that antenatal diagnosis, with its attendant risks, was available. Meanwhile another maternal blood test showed microcytosis but failed to confirm a raised HbA_2 value. This suggested that the patient was either a 'silent carrier' of β-thalassaemia trait or had an α_1 thalassaemia trait, which would not have been an indication for antenatal diagnosis under her circumstances (Weatherall and Clegg, 1972).

However, these results became known after she had elected to seek an antenatal diagnosis and a two litre blood transfusion had been given, a customary procedure at this centre aimed at clearing reticulocytes from the maternal blood and thus simplifying the interpretation of mixed samples of fetal and maternal blood (Fairweather *et al*, 1978). Because of the uncertainty as to the exact diagnosis, four weeks had to elapse before biosynthetic studies confirmed that the patient was a 'silent β-thalassaemia carrier'. In the normal individual the ratio of synthesis of the α and β chains of HbA = 1 (range 0·9 to 1·1 in our laboratory). In β-thalassaemia trait, β-chain synthesis is reduced and the $\frac{\beta}{\alpha}$ ratio is about 0·5 (range 0·37 to 0·75 in our laboratory). In α-thalassaemia trait, α-chain synthesis is reduced and the $\frac{\beta}{\alpha}$ ratio is more than 1 (range 1·2 to 1·5 in our laboratory). At the time of presentation, the patient's $\frac{\beta}{\alpha}$ ratio was 0·39 indicating β-thalassaemia trait even though this had not been signalled by a raised HbA_2 level; thus she was a 'silent carrier' of β-thalassaemia trait. Nine months later, these findings were confirmed: the $\frac{\beta}{\alpha}$ ratio was again 0·4 and the HbA_2 level was again normal.

Direct measurement of the α globin gene in the DNA from maternal leucocytes provided no evidence of α-thalassaemia (Ottolenghi *et al*, 1974; Kan *et al*, 1974).

Despite the delay, the patient asked that antenatal diagnosis should be undertaken and a further blood transfusion was given. At 22 weeks gestation, ultrasound examination showed a twin pregnancy with a transverse septum and a posterior placenta. A Dyonics needlescope was inserted into the superior sac under local anaesthesia, but maternal bleeding into the amniotic fluid obliterated any view of the chorionic plate. Blind needling at a different site produced 100 per cent fetal blood at the second attempt. The three subsequent samples of amniotic fluid taken from this site showed 80 to 90 per cent fetal cells; 15 to 20 per cent fetal cells; and finally only a trace of fetal cells, indicating cessation of fetal bleeding. One ml of methylene blue was injected into the superior sac before removal of the needle.

The objective of blind needling is to make a fetal vessel on the chorionic plate bleed into the amniotic fluid and small samples (0·1 to 0·5 ml) of blood stained amniotic fluid are then withdrawn. These are examined immediately using the Coulter Channelyser for the presence of fetal cells (Fairweather *et al*, 1978). Most contain only maternal cells; sampling is stopped as soon as a specimen containing sufficient fetal cells has been obtained.

At this stage the patient had to empty her bladder so that rescreening was necessary. The lower twin was still transverse but the placenta was now entirely anterior. Needling of the second sac was undertaken with a 19 gauge spinal needle and clear amniotic fluid aspirated. Blind needling of the chorionic plate was successful with 10 per cent fetal cells in the first sample, but a larger sample was considered desirable. In all, three further attempts at needling the chorionic plate were necessary (4 to 5 samples of amniotic fluid taken with each). Sample 14 contained 100 per cent fetal cells and the two subsequent samples had 20 per cent and 10 per cent fetal cells respectively, indicating cessation of fetal bleeding. At the conclusion of the procedure the real time scanner showed two normal fetal heart rates.

The amount of fetal blood obtained in the samples was 0·5 ml from twin I and 0·3 ml from twin II: 0·1 ml of each was used for the diagnosis and the rest sent for extended blood grouping. The results obtained three days later (*vide infra*) suggested that both fetuses were normal or had thalassaemia trait, but excluded thalassaemia major. The pregnancy continued normally until premature labour started at 30 weeks. The patient was delivered vaginally of male twins weighing 1·2 kg and 1·6 kg. Both required phototherapy for hyperbilirubinaemia and the second twin required a top-up transfusion. Examination of the placenta showed that the twins were monozygotic.

Results of blood tests

On extended screening of antenatal fetal blood samples, both twins were shown to have identical blood groups, and this was confirmed postnatally.

The fetal blood samples gave $\frac{\beta}{\gamma}$ ratios of 0·051 and 0·042 respectively. These levels are in the overlap between the normal and the thalassaemia trait ranges.

At birth the $\frac{\beta}{\gamma}$ ratios were 0·133 (twin I) and

0·129 (twin II). Normally the ratio is 1 at term but in premature infants the above ratios are probably normal. Both twins had chain balance suggesting normality, and these results definitely excluded thalassaemia major.

At follow-up (14 weeks, or the equivalent of one month of age had the infants been born at term) the $\frac{\beta}{\gamma}$ ratios were 1·5 (twin I) and 1·6 (twin II) again with normal chain balance, suggesting normality rather than a β-thalassaemia trait, the distinction between the two being very difficult in the neonatal period. At 10 months the haematology of both twins was normal, and the $\frac{\beta}{\alpha}$ ratios were 1·25 (twin I) and 1·0 (twin II), confirming normality.

DISCUSSION

Despite the probable increased risks inherent in antenatal diagnosis in a twin pregnancy, this case report shows that with ultrasound guidance it is feasible to obtain fetal blood samples from both gestational sacs. Indeed, we believe it is particularly important to attempt the diagnosis in a twin pregnancy since the birth of two children with thalassaemia would be a signal disaster. We discussed beforehand with the mother what her attitude would be to the two problems we anticipated, namely obtaining a diagnosis in one fetus only, or finding one normal and one thalassaemic fetus. She declared she would not contemplate the abortion of a normal fetus for the sake of an affected one. Because the incidence of identical twins amongst Cypriots is 50 per cent, the calculated risks were as follows: both fetuses having thalassaemia, 1 in 8; one having thalassaemia and one being normal, 2 in 8; and both being normal or only carriers, 5 in 8. If we could diagnose only one fetus there was a 50 per cent chance that the second diagnosis would be the same, and we decided to make the attempt since even such incomplete information would be helpful to the mother.

The bleeding produced by the needlescope was probably a direct result of placental trauma, and is one of the limiting factors in using this instrument. Two other features of the procedure were noteworthy. Firstly, the injection of methylene blue into the first sac sampled was useful in confirming entry into the second sac. Secondly, with regard to the apparent change in placental site, we have observed this on many occasions particularly when a full bladder is emptied; it is probably due to rotation of the uterus on its longitudinal axis. It is unreasonable to blame the onset of premature labour eight weeks after fetal blood sampling on the procedure, as premature labour is common with twin pregnancies.

This case report also illustrates other features of the antenatal diagnosis of β-thalassaemia. Firstly, an accurate diagnosis of the β-thalassaemia carrier state in both parents is essential for a rational decision about the need for antenatal fetal blood sampling. The commonest differential diagnosis is between β-thalassaemia trait, characterized by microcytosis and raised HbA_2 which occurs in 15 per cent of Cypriots, and α_1 thalassaemia trait characterized by microcytosis and a normal or reduced HbA_2 which occurs in 2 to 3 per cent of Cypriots. There is no risk to the fetus of serious disease if one parent has α_1 and the other β-thalassaemia trait. However, 'silent' β-thalassaemia in which either the HbA_2 is normal or there is no microcytosis does sometimes occur and must be excluded by studies of haemoglobin biosynthesis if an individual with apparent α_1 thalassaemia trait is married to one with typical β-thalassaemia trait.

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BOOK REVIEWS

Gynecologic and Obstetric Urology. Edited by H. J. Buchsbaum and J. D. Schmidt. Pp xiv+461 with illustrations. W. B. Saunders, Philadelphia, 1978. Price: £24.00.

There is an awareness that the expansion of knowledge and information is encouraging specialization within the broad and conventional disciplines of medicine. For many years the training of a gynaecologist in the States had contained a programme of urology, guided by the example of Howard Kelly at Johns Hopkins Medical School. Nowadays this is less evident and similarly, there is little exposure to gynaecology in the urologist's post-graduate training. This book, edited by a gynaecologist and a urologist, is an attempt to correct this: it gives extensive coverage of gynaecological urology and of the urinary tract in pregnancy, and successfully demonstrates the interdependence of urology and gynaecology. The majority of contributors are gynaecologists, some of whom have already made major contributions in this field. The organisation of the book has been thoughtful and the inclusion of chapters on post-operative bladder drainage, urinary tract infection and involvement of the urinary tract in benign and malignant disease is praiseworthy. Repetition of information with so many contributors is difficult to avoid, and this has occurred in several places, e.g. anatomy of the lower urinary tract, repair of vesico-vaginal fistula. I would like to have seen a wider description of the anatomy and physiology of the mechanism of continence and more detail of urodynamic investigative techniques. In a work as comprehensive as this, there is room for chapters on drug action, acute and chronic retention, and the psychiatric implications of bladder and urethral disorders. The book is well illustrated and each chapter is followed by references, some of which have been supplanted by more recent publications. These few criticisms should not detract from the overall high standard of the book.

S. L. Stanton

Viral Diseases of the Fetus and Newborn. Vol. XVII in the Series Major Problems in Clinical Pediatrics. By J. B. Hanshaw and J. A. Dudgeon. Pp xvi+356 with illustrations. W. B. Saunders Company Ltd, Philadelphia, 1978. Price: £14.00.

It is a privilege to be asked to review a book of such outstanding quality and which, I have no doubt, will be of immediate and lasting value to many people. Here is a comprehensive account of present knowledge of fetal and neonatal viral diseases presented by two leading workers in the field. Detailed chapters relate to each of the viruses known, or thought, to be associated with perinatal infection. In addition there are sections on pathogenesis, immunology, differential diagnosis, laboratory diagnosis and prevention and treatment. Each of the fifteen chapters incorporates an extensive reference list and the illustrations are well-chosen and complement the text. The book will be welcomed by obstetricians, paediatricians and clinical microbiologists and in addition it will be of benefit to others concerned with infant care—in particular audiologists and nursing officers. The price is not excessive and I hope it will find a place on the bookshelves of the general practitioner and clinical student.

D. J. Jeffries

Realities in Childbearing. By Mary Lou Moore. Pp ix+772 with illustrations. Holt-Saunders Ltd, Eastbourne, 1978. Price: £10.50.

The rather ambiguous title is explained in the opening chapter under the sub-heading 'Reality versus the Myth'. Although in many ways this is an interesting book, the content and presentation are too advanced for a student learning about obstetrics for the first time. The book is more appropriate to the midwife who has a good background knowledge of basic obstetrics and wishes to advance that knowledge. The role of the American nurse-midwife is very different from that of the British midwife, so that much of what is written in relation to the former is not relevant to the latter. However, many comments are relevant, for example, 'nurses have found the need to increase their skills in assessment, in communication, and in support of families . . . and also to increase their skills in technical areas', is as true on this side of the Atlantic even though it has a somewhat different meaning. Nor could one argue with the knowledge considered necessary to adequately care for childbearing families as outlined in Chapter 1. I sympathize with the philosophy of the book and as a teacher share the author's obvious enthusiasm for wanting the student to enjoy the subject as she so obviously does. However the text is rather confusing and difficult to follow at times. Some subjects are dealt with in great detail e.g. psychological aspects, and others rather briefly, e.g. medical disorders associated with pregnancy. In some cases definitions and terminology would not be acceptable in this country, para is referred to as the number of pregnancies beyond twenty weeks. There are extensive references at the end of each chapter although with the exception of Chapter 7 there is no indication in the text to what they refer. I found it difficult to evaluate a book so completely orientated to the American nurse. It is of limited value to the British midwife, although the chapters dealing with psychosocial and cultural aspects of childbearing are excellent and useful.

M. A. Hickman

Birth. By Judy Chock and Margaret Miner. Pp viii+168 with numerous photographs. Thomas Y. Crowell Co Inc, New York, 1978. Price: £7.95.

At first glance this appears to be the classic 'coffee-table' book of birth, and indeed, the format has the glossy texture of popular picture book presentations. There are nine chapters covering the antenatal period, labour, birth and lying-in, but the book also covers common areas of abnormal obstetrics such as Caesarean section, forceps delivery and multiple births. The book is written for pregnant women as an educational manual and not as a text book for doctors and midwives. The pictures are superb both in monochrome and colour and there is a good balance between explicit photographic illustrations and the written text. Furthermore the text is sensible, factual and unbiased. The authors present a variety of views currently in circulation without taking sides about their respective merits. At the same time they do not attempt to conceal the more difficult aspects of child-bearing. If there is a reservation about this book, it is that it is written at a level that will presuppose a substantial degree of literary and basic knowledge. The book should be very successful. The price is modest and it fills a gap in a market which is replete with books of fantasy rather than comforting reality.

E. M. Symonds

Septic Shock in Obstetrics and Gynecology. (Vol. II in the Series: Major Problems in Obstetrics and Gynecology). By D. Cavanagh, P. S. Rao and M. R. Comas. Pp xi+135 with illustrations. W. B. Saunders Company, Philadelphia, London and Toronto. Price: £9.50.

In many countries septic shock is still a common and important condition demanding a high standard of medical care and yet often resulting in maternal death. This book is a small monograph on the subject compiled by three authors who have made major contributions in the study of this condition. It is divided into seven chapters and covers all aspects of the pathogenesis, clinical features and management of endotoxic shock. There is a particularly good chapter on pathogenesis which provides not only a comprehensive survey of current views of all aspects of the subject but also provides an excellent bibliography of some three hundred references. Specific instructions are provided on monitoring and clinical management and at a time when the problem is diminishing in frequency, it is valuable to have available guidelines which provide a recipe for safe care. This is not a book that one could recommend as a necessary purchase for all gynaecologists. It is a monograph that will have a useful place on the bookshelves of medical libraries and in particular, hospital libraries.

E. M. Symonds

Neonatal Medicine. By Malcolm L. Chiswick. Pp 101 with 106 figs and 56 tables. Update Publications Limited, London, 1978. Price: £3.95.

Neonatal Medicine is a simple, readable, well illustrated introductory text on neonatal care. After two introductory chapters, the first on fetal assessment, the second on adjustments to extra-uterine life, there are separate chapters on examining the newborn, problems of the low birth weight infant, nutrition, infection, jaundice, respiratory problems, neurological problems, gastro-enterological problems, genito-urinary problems, metabolic adaptions to extra-uterine life and the final thirteenth chapter on modern concepts of maternal and infant care. The book is very well presented and written in a clear, succinct style. It gives a balanced account of current clinical practice. The text is enlivened by comments which reflect the author's considerable clinical experience. The book is published by Update Publications and maintains the high standard of their journal. Figures, diagrams and colour prints are beautifully presented, only occasionally is colour used for colour sake or information forced into inappropriate diagrams. Coloured clinical photographs abound and in general are very helpful. Personally I would have avoided Post Mortem photographs and there is not a need for three pictures of Potter's syndrome. Differential diagnoses are clearly presented in table form. These usually help the text although some, for example the one on abnormal neurological behaviour, have perhaps the opposite effect of what was intended. I would strongly recommend this book to all those approaching neonatal medicine for the first time. The authors and the publishers are to be congratulated on producing such an excellent book at such a reasonable price.

D. Hull

1978 Year Book of Obstetrics and Gynecology. Edited by ROY M. PITKIN. Pp 480 with illustrations. Year Book Medical Publishers Ltd, London, 1978. Price: £18.00.

As in the previous volume, the 1978 Year Book starts with a 'Current Literature Quiz' the pages of the answers to which precede the index. Surprisingly the subject of the long-term effects of stilboestrol on the male fetus is not included in the quiz. The general arrangement of subjects is as before with sexual problems reviewed in the section headed Primary Care and Public Health. The one special long article is by Linzey and Freeman, from Long Beach, California, on antepartum fetal monitoring. One of their conclusions is that at present the oxytocin challenge test is preferable to unstressed monitoring. The editor of a Year Book has the difficult task of combining vision and caution in his comments on individual papers extracted. Dr Pitkin has carried on well the tradition of DeLee and Greenhill, even if he confesses that before 1977 he had not heard of the practice of infibulation.

S. Bender

Adolescent Obstetrics and Gynecology. Edited by KREUTNER and HOLLINGSWORTH. Pp 658 with illustrations. Year Book Medical Publishers Inc, London, 1978. £32.50.

THE title of this book Adolescent Obstetrics and Gynaecology does not give a correct impression of its contents. True it deals with obstetrical and gynaecological aspects of that period of life but considerable emphasis is laid on problems of adolescent sexuality with less space than seems appropriate being devoted to clinical disorders—especially gynaecological ones. For example half a page is given to the pregnant adolescent with heart disease, rather less than two pages to dysfunctional uterine bleeding and there is no consideration at all of malformations such as haematocolpos and congenital absence of the vagina which present at that time; by contrast a chapter of 35 pages is devoted to the adolescent rape victim. Some sections are useful as aspects of the discipline not often given detailed consideration elsewhere; such as drugs and the adolescent including the pregnant adolescent and the mentally retarded adolescent. In other more conventional areas, however, the extent and detail of the coverage of certain important abnormalities leaves something to be desired and generalizations which are not always strictly accurate are too often resorted to. All in all if the book is read for its clinical, obstetrical and gynaecological content it will probably be found to be disappointing; if it is read for its insight into aspects of adolescent behaviour, especially sexual behaviour, it will be much more satisfying.

Sir John Dewhurst

The Cesarean Birth Experience. By BONNIE DONOVAN. Pp xv+240 with figs and tables. Beacon Press, Boston, 1978. Price: £5.50/£2.50.

THIS book is written by a woman who has had both elective and emergency Caesarean sections and now runs courses to instruct and prepare patients. The aim of the book is to be a 'complete and reassuring guide for patients and professionals'. The first five chapters discuss the pregnancy, indications for Caesarean section and the various monitoring techniques that may be performed. The later chapters describe the procedures used during admission, surgery and the puerperium, and there are many revealing comments. Much time is spent in arguing the case for performing the operation and regional anaesthesia, principally so that 'visual bonding' may occur early. The need for the husband to be present during the operation is explained. However, the author admits that some hospitals may not agree to this and discusses how pressure may be exerted to achieve this objective. A list of useful questions for the patient to ask in selecting both obstetrician and hospital, is given. Undoubtedly many women anticipating normal delivery are disappointed at having a Caesarean section. This book attempts to reassure and explain the situation to them. However, indications given for surgery are so technical that many patients would not understand and presumably professionals would refer to their own textbooks. The photographs of mother and baby progressing through the theatre are badly reproduced and difficult to see. The historical chapter is interesting but makes no comment on the increased safety of anaesthetics. A great deal of jargon is used, making the book difficult to read.

W. Love

Essential Exercises for the Childbearing Year. By ELIZABETH NOBLE. Pp 180 with numerous illustrations. John Murray Publishers Ltd, London, 1978. Price £2.95.

THE author of this excellent book is an Australian trained physiotherapist who is now working in the States. She sets out to teach women why, and how to care for their bodies during pregnancy, labour and the 3 months post-partum period.

The book contains chapters on the need for exercise during pregnancy, the abdominal and pelvic floor muscles, posture and comfort during childbearing, relaxation and breathing during normal labour and ends with a chapter on coping with a Caesarean birth. The anatomy and function of all the muscle groups mentioned are well described and clearly illustrated. The exercises chosen are simple and comparatively few in number, they are well described and all the exercises and postures are clearly illustrated by nude figures. The book is so good and so comprehensive—it runs to 160 pages—that criticism is difficult but there are some points at variance with common practice here. For example, great attention is paid to relaxation of the pelvic floor during pushing in the second stage of labour, to facilitate this mothers are advised to breathe out very gently while retracting their abdominal wall. It is difficult to see how adequate fixation of the diaphragm can be achieved in this way. Also the author suggests 'bridging' as an early postnatal exercise to improve the circulation and stability of the back of the pelvis and does not seem to be aware of the possible danger of air embolism when the hips are raised above the heels in the horizontal position. Mrs Noble makes out a convincing case for specific exercises during the childbearing year in addition to daily activity. Both obstetricians and students might be interested to read her book so that they are aware what they are committing their patients to when they refer them to an obstetric physiotherapist. If such a physiotherapist is not available they might be glad to recommend the book to their more intelligent patients.

Lady Margaret Williams

Reproductive Endocrinology: Physiology, Pathophysiology and Clinical Management. Edited by SAMUEL S. C. YEN and ROBERT B. JAFFE. Pp xiii+579 with figs and tables. Holt-Saunders Ltd, Eastbourne, 1978. Price: £28.00.

IT is probably the perfect moment for an encyclopedic work on clinical and experimental reproductive endocrinology. Five years ago we were in the midst of an explosive growth of knowledge on the hypothalamic and pituitary hormones. Given the time-lag for the production of a text book there was every chance that large sections would be outdated by the time it was in print. Today that growth has ceased; there are no wholly new concepts, such as that of human prolactin or the characterization of releasing factors, and current work is almost entirely of the nature of a tidying-up operation. Perhaps the only exception is the pineal gland which has justly been termed the 'last frontier' of endocrinology. This very under-rated organ claims but a single page of the present text. The editors have put together a team of authors which could not be bettered given their exclusively North American origin. Each has provided an in-depth review of a specified area. The coverage of gynaecological endocrinology is very complete, ranging from the most fundamental aspects of biochemistry and physiology to a very useful set of detailed protocols on the performance of dynamic function tests. Diagnosis and clinical management are discussed at great length, and if the conclusions are not always in line with accepted European practice this is more a matter of opinion than of basic principle. The major flaw in the book is the coverage of obstetric endocrinology which is relegated to two short chapters at the end of the book. Though excellent in themselves, the scope is very limited: virtually no mention is made of the endocrinology of parturition or of the clinical use of placental function tests. This section should have been expanded or left out. The publishers and editors should be congratulated on the superb quality of presentation, from the half-tone plates to the figures and graphs, many of which have been enhanced by the use of two colour tones. Their labours have been worthwhile—this is undoubtedly the best available text on gynaecological endocrinology and should find a place on the shelves of every clinician or department with any claim to specialize in this important field of clinical medicine. The price is modest for a text of such practical value and elegance.

T. Chard

British Journal of Obstetrics and Gynaecology
March 1979. Vol. **86.** p 256

CORRESPONDENCE

Secretion Patterns of Plasma-progesterone, 17-Hydroxyprogesterone and 20α Hydroxypregn-4-en-3-one in Early Abnormal Pregnancy. Harrison, R. F., Youssefnejadian, E., Brodovcky, H., Johnson, M., and Dewhurst, Sir John (1978): *British Journal of Obstetrics and Gynaecology*, **85**, 927.

From: K. Dalton.

Sir,

One wonders why Harrison *et al* used norethisterone and medroxyprogesterone for this study, especially in view of the finding that these synthetic progestogens produce a significant depression of the plasma progesterone levels in the luteal phase in normal menstruating women (Johansson, 1971). It is possible that their use may account for the low hormone levels observed in patients D, E and F. Furthermore, the use of these progestogens is contra-indicated because of the risk of masculinization in the female fetus. Would it not have been safer and more effective to use progesterone intramuscularly or by suppositories, for this is known to raise the plasma progesterone levels (Nillius and Johansson, 1971) without risk to the fetus?

No details are given of the presence or absence of pregnancy symptoms (nausea and vomiting, headaches and tiredness) which may indicate those patients likely to benefit from progesterone supplement (Dalton, 1977) and those whose abortions are due to chromosomal abnormalities.

Yours etc,
KATHARINA DALTON
46 Harley Street
London W1N 1AE

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Dalton, K. (1977): *Premenstrual Syndrome and Progesterone Therapy*. William Heinemann Medical Books, London, p 123.

Johansson, E. D. B. (1971): *Acta endocrinology* (Kobenhaven) **68**, 799.

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2. Symposium on Treatment of Foetal Risk, Baden, Austria, May 1972
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British Journal of Obstetrics and Gynaecology

EDITOR: FRANK E. LOEFFLER

SUPPLEMENT No. 3

A PROSPECTIVE, CONTROLLED TRIAL OF SIX FORMS OF HORMONE REPLACEMENT THERAPY GIVEN TO POSTMENOPAUSAL WOMEN

T. Lind, E. C. Cameron, W. M. Hunter, C. Leon, P. F. Moran
A. Oxley, J. Gerrard and U. C. G. Lind

Supplement Editor: Sonja Chayen

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A PROSPECTIVE, CONTROLLED TRIAL OF SIX FORMS OF HORMONE REPLACEMENT THERAPY GIVEN TO POSTMENOPAUSAL WOMEN

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A PROSPECTIVE, CONTROLLED TRIAL OF SIX FORMS OF HORMONE REPLACEMENT THERAPY GIVEN TO POSTMENOPAUSAL WOMEN

BY

T. Lind, E. C. Cameron, W. M. Hunter, C. Leon, P. F. Moran, A. Oxley, J. Gerrard and U. C. G. Lind

SUMMARY

This study was undertaken to investigate the effect of various forms of hormone replacement therapy (HRT) upon postmenopausal women while controlling as many variables as possible. It was felt that the age, duration of amenorrhoea and the general health of the patients should be as comparable as possible and that each patient should provide her own pretherapy and post-therapy control data. In addition, it was felt that any placebo effect should be investigated and the patients were therefore randomly allocated to placebo tablets or one of six available forms of HRT.

The age/sex registers of two large general practices were scrutinized and all women between 49 and 54 years of age were asked to cooperate; for a variety of reasons only 56 women were suitable and willing to take part in the project, yielding 8 women for each of the seven possible therapy groups. Blood samples were taken at 7-day intervals three times before therapy was given and the mean of the three values was used as the control value. The women returned on day 21 of each subsequent therapy cycle for six consecutive months and finally three months after discontinuing therapy.

From the data the following broad conclusions can be drawn: (i) some women have classic symptoms of hot flushes and sweating despite high endogenous oestrogen concentrations; (ii) vaginal cytology is a relatively poor indicator of endogenous oestrogen status; (iii) while follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations are reduced on HRT neither is decreased to anywhere near premenopausal values while prolactin is unaffected; (iv) plasma cholesterol levels are reduced on HRT, the pulse rate is slower and both systolic and diastolic blood pressure are reduced to a small but significant extent; (v) there is no adverse effect upon blood clotting; and (vi) most women experience significant or complete relief of symptoms on all forms of HRT as do some women taking a placebo.

The combined preparations containing an oestrogen and progestogen produced vaginal bleeding in only 80 per cent of the women. Thus protection by regular endometrical shedding may not be afforded to all women. As vaginal bleeding is unacceptable to most women if they can achieve the same symptomatic relief without inducing menstruation, it is suggested that women have a low dose oestrogen preparation prescribed cyclically for 6 to 12 months. If therapy is to be maintained for a longer time, uterine curretage should be undertaken at regular intervals to exclude the possibility of endometrial carcinoma developing.

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INTRODUCTION

Possible benefits and drawbacks of hormone replacement therapy (HRT) for postmenopausal women have been widely debated. A comprehensive review of all of the available literature would be difficult but the reports of international symposia held in London during 1975, La Grande Motte in France during 1976 and Sheffield during 1978 demonstrate the spectrum of current opinion (Campbell, 1976; Van Keep *et al*, 1976; Cooke, 1978). Difficulties can arise in the interpretation of data from HRT studies: sometimes synthetic oestrogen compounds are compared with 'natural' oestrogens (e.g. Jones *et al*, 1977); women of widely different ages may be compared as in the study of 60 women aged 49 to 91 years reported by Chakravarti *et al* (1976); when a 'no-therapy' control group is used, the women may be different to those given therapy rather than the same women before therapy (e.g. Stangel *et al*, 1977). If symptomatic responses are being investigated, the motivation of women taking part must be carefully considered; women who spontaneously attend an open 'menopause clinic' must be regarded as highly selected.

The present study was undertaken to investigate the effect of various forms of HRT upon postmenopausal women, with control of as many variables as possible. It was felt that the age, duration of amenorrhoea and general health of the patients should be as comparable as possible and that each patient should provide her own pretherapy and post-therapy control data. In addition the patients should be randomized as to whether they received a placebo tablet or one of the six possible forms of HRT detailed below.

PATIENT SELECTION

Age

It seemed unreasonable to compare the responses of women of markedly different ages to HRT; a woman of 70 years of age with perhaps 20 years amenorrhoea and a woman aged 55 years and five years amenorrhoea are both 'postmenopausal' yet are unlikely to demonstrate the same degree of response to therapy. Our patients were between 49 and 54 years of age.

Duration of amenorrhoea

Similar constraints seemed necessary with regard to the duration of amenorrhoea; only women with amenorrhoea for not less than six months and not more than 48 months were accepted.

Reasons for exclusion

Women already taking hormone preparations, those suffering from established, chronic illnesses, and those with a known psychiatric history were excluded. Women who had had a hysterectomy were also excluded because of the need to determine the proportion of women in whom menstruation was induced by HRT.

Method of patient recruitment

The age/sex registers of two local general medical practices were scrutinized and women were selected by their date of birth as falling within the chosen age range; whether they had reached the menopause was not usually known and a questionnaire was sent to each enclosing a prepaid, addressed envelope for reply. A letter was enclosed explaining the purpose of the research, and it was made clear that if a more detailed explanation would be helpful, a female member of staff was willing to visit them at home at a convenient time and date. A car service would be provided to collect them from home and return them on every occasion. In this way, it was hoped to achieve maximum patient cooperation particularly as current publicity in the newspapers, womens' magazines and on television had all been favourable to the use of HRT. It was requested that the questionnaire be returned even if the person could not help, as a check that everyone had been successfully contacted.

The reponse was interesting; in one practice there were 382 women of the appropriate age but only 232 (61 per cent) replied. In the second practice, of 116 potentially suitable patients only 61 (53 per cent) replied (Table I). It could be argued that the questionnaire was not sufficiently clear in explaining matters but each person was asked to return the questionnaire in the prepaid envelope yet this was not used to ask for more information or even to indicate that the woman was unwilling or unable to help. Many women of this age have returned to full time working and this could be a deterrent to helping with research; however, of those ultimately recruited, 54 per cent were working full-time, 18 per cent were working part-time and 28 per cent were housewives.

Of the 232 women who replied from the first practice, 100 (43 per cent) were still menstruating and therefore unsuitable for our study; of these, 76 would have been willing to help and 24 would not. From the remaining 132 who were post-menopausal, 45 (19 per cent) declined to help, 38 (16 per cent) were willing to help but their duration of amenorrhoea fell outside our defined limits, leaving only 49 (21 per cent) suitable volunteers from the whole group. These data and the equivalent figures for the second practice are given in Table I, showing that only 65 suitable and cooperative patients were recruited from the total of 498 women contacted. For domestic reasons nine of these women were unable to take part in the study when the full scheme was explained, leaving a final group of 56.

METHODS

Standardization of conditions

It was hoped that seeing the patients in a fasted or 'basal' state on each occasion would reduce within-patient and between-patient variability. Patients therefore attended the unit between 0900 and 0930 hours having fasted overnight for at least 12 hours. The majority were collected from their homes by a staff member; others came in their own cars. After the patient had been sitting quietly at rest for 20 to 30 minutes, pulse and blood pressure were recorded twice on a recording sphygmomanometer (Erlag) after which blood was drawn from an antecubital vein for the various determinations. Each woman had a blood sample taken at 7-day intervals three times before being given any therapy and the mean of these three values was used as the control value. They returned on day 21 of each subsequent therapy cycle for six consecutive months and finally three

TABLE I

Criteria used in patient selection, the numbers in each medical practice who were of the appropriate age range, and the responses of the women who replied

Aged: 49-54 years   Duration of amenorrhoea: 6–48 months
Exclusions: those taking hormones, having chronic illnesses, previous hysterectomy or psychiatric history

	Practice 1	Practice 2	Both
Approached:	382	116	498
Replied:	232 (61%)	61 (53%)	293
(a) Still menstruating:			
No	24	4	28
Yes	76	17	93
	100	21	121
(b) Post-mens; but outside range			
No	45	12	57
Yes	38	12	50
	83	24	107
(c) Acceptable	49	16	65
Total replies	232	61	293

'Yes' and 'No' refer to willingness or otherwise to cooperate in the study.

months after discontinuing all therapy (see details below).

Laboratory determinations

The factors which were measured at each visit are listed in Tables II and III. The methods used were as follows:

Luteinizing hormone (LH) and follicle stimulating hormone (FSH). Highly specific antisera (F 87 and M 93 respectively) were kindly supplied by Professor W. R. Butt of the Birmingham and Midland Hospital for Women. Assay methods were adapted for this study from those described by Hunter and Bennie (1979) to yield working ranges of 2.7 to 280 (LH) and 4.0 to 160 (FSH) mU/1 serum in four hours total incubation time at room temperature followed by 16 hours incubation at 4°C for the double antibody separation. This separation was performed by an automated system developed by Professor K. D. Bagshawe's laboratory at Charing Cross Hospital, London. The standards used were from the National Institute of Biological Standards and Control: for LH 68/40 assumed 77 U/ampoule and for FSH 69/104 assumed 24 U/ampoule. The within-batch coefficients of variation (CV) were 4.9 per cent for LH and 6.5 per cent for FSH (n=10) and the between-batch CV were 8.1 per cent and 7.8 per cent respectively (n=10).

Oestrone and oestradiol. The radioimmunoassay for oestrone used anti-E_1-6-(O-carboxymethyl) oximino-bovine serum albumin (BAS) serum with a (2,4,6,7-$^3H_4$)-E tracer (Specific activity 90 Ci/mmol; Radiochemical Centre, Amersham, England). The within-batch CV was 9.2 per cent and the between-batch CV was 11.3 per cent. Oestradiol was measured by a method modified from Cameron and Jones

TABLE II

The observations made and samples taken at each visit; the measurements determined on the blood sample are listed in Table III

Observations made and samples taken	Visits									
	Pretreatment			Treatment						Post-treatment
	1	2	3	4	5	6	7	8	9	10
Weight	✓	✓		✓	✓	✓	✓	✓	✓	✓
Pulse	✓		✓	✓	✓	✓	✓	✓	✓	✓
Blood pressure	✓		✓	✓	✓	✓	✓	✓	✓	✓
Blood sample	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urine (culture)	✓		✓	✓	✓	✓	*	*	*	✓
Vaginal smear	✓		✓	✓	✓	✓			✓	✓

* Urine only cultured if previous samples infected.

TABLE III

Laboratory measurements performed on each blood sample

FHS	Cholesterol	Prothrombin time
LH	Triglyceride	PTTK
Oestradiol	Urea	FDP
Oestrone	Creatinine	*Anti-thrombin III
Progesterone	Fasting blood glucose	Fibrinogen
Progestogen		Factors V, VII, VIII and X
*Prolactin		Platelet count
Full blood count (Coulter)		Platelet Agg (ADP and collagen)

* Not on every patient.
Progestogen: Norethisterone or norgestrel.
PTTK: Partial thromboplastin time with Kaolin.
FDP: Fibrinogen degradation products.
Platelet Agg: Platelet aggregation studies.

(1972), using an anti-E_2-6-(O-carboxymethyl) oximino-bovine serum albumin (BSA) serum with a (2,4,6,7-$^3H_4$)-E_2 (specific activity, 84 Ci/mmol; Radiochemical Centre, Amersham, England). The within-batch CV was 8.3 per cent and the between-batch CV was 10.9 per cent.

Progesterone was determined by the radioimmunoassay method described by Dighe and Hunter (1974) using a solid phase antibody and (1,2,6,7-^3H) progesterone (specific radioactivity 80-110 Ci/mmol). The between-batch CV was 9 per cent (n=10).

Norethisterone was measured by the method of Morris and Cameron (1975) using an anti-norethisterone-11α-bovine serum albumin serum and a (15,16-$^3H_2$)-norethisterone tracer (specific activity 20 Ci/mmol; NEN GmbH, Frankfurt am Main, Germany). The within-batch CV was 8 per cent and the between-batch CV was 13.6 per cent.

L-norgestrel was assayed by the method of Cameron and Cameron (in preparation) using an anti-norgestrel-11α-BSA serum and a (15,16-$^3H_2$)-norgestrel tracer (specific activity 57 Ci/mmol; a gift from Scherling AC, Berlin, Germany. The within-batch CV was 6.4 per cent and the between-batch CV was 10 per cent.

Prolactin was determined by the immunoassay method of Hwang et al (1971) modified for use with the Kemtech 3000 automated radioimmunoassay system described by Bagshawe (1975). The reagents, originally obtained from Professor Friesen, were made available to us by Dr A. S. McNeilly (Edinburgh). The specific activity of the labelled hormone was 150 μCi/μg, the reference range 25 to 360mU/l and reference standard MRC 77/222. The within batch CV was 5.5 per cent (n = 10) and the between-batch CV was 13.5 per cent (n = 11).

Cholesterol and triglyceride. The blood was withdrawn as rapidly as possible by single venepuncture with minimum stasis and was put into a plain glass tube which had been kept in an ice bath. The tube was returned to the ice bath until a firm clot had formed and the resultant serum was assayed the same day for cholesterol and triglyceride by the method of Eggstein and Kreutz (1966) using reagents supplied by Boehringer Corporation (London) Ltd.

Urea and creatinine were determined the day the samples were taken. Urea was measured by Chaney and Marbach's (1962) modification of Berthelot's reaction; creatinine was measured by a kinetic technique using an LKB 8600 reaction rate analyser as described by Bierens de Haan (1972).

Plasma glucose was measured on the same day by a modification of the glucose oxidase-peroxidase enzyme technique (Trinder, 1969).

Antithrombin III. Because of a 'bewildering variety of techniques' (Leading article, 1976) which can be used to determine this factor, three assay methods were used for each sample: (i) Lane *et al* (1974); (ii) Howie *et al.* (1973); (iii) a single radial immunodiffusion technique using antithrombin III plates commercially available from Hoechst Pharmaceuticals.

Fibrinogen was determined by the heat precipitation method of Foster *et al* (1959).

Prothrombin time (PT). The one stage prothrombin time was measured by a technique based on the original method of Quick (1957).

Partial thromboplastin time with kaolin (PTTK). This was measured by the method described by Biggs (1976) using Folch's phospholipid as platelet substitute.

Fibrinogen degradation products (FDP) were measured using the standard Burroughs-Wellcome Thrombo-WellcoTest.

Factor analysis. In the case of Factors V, VII and X activity, a one-stage assay was used in which a normal control plasma was added to a substrate plasma, deficient in the factor being determined, and compared to the effect of the sample under review on the same deficient plasma using the Quick prothrombin time. Deficient plasma was obtained from American Hospital Supplies. Factor VIII activity was measured using the one-stage method of Breckenridge and Patnoff (1962). The Factor VIII standard was obtained from Immuno-Diagnostica and contained one unit of Factor VIII activity/ml.

Platelet count. This was measured on a Thrombocounter (Coulter Electronics Ltd.)

Platelet aggregation studies. These were performed on an EEL 169 platelet aggregation meter connected to a Servoscribe S potentiometric recorder. Aggregation with adenosine diphosphate (ADP) was by the method published in Broadsheet 83 (1975) of the Association of Clinical Pathologists after the method of Born (1962). The collagen for aggregation studies was obtained from Hormon-Chemie, München and used at a final concentration of 4μ/ml.

Vaginal cytology. After passing a bi-valve speculum, two vaginal smears were obtained by drawing a disposable wooden spatula down the full length of each lateral vaginal wall. After fixation, these slides were stained by a Romanowsky staining method. Approximately 500 cells were counted per slide and the basal, precornified and cornified cells expressed as a percentage of the total number counted.

Urine culture. All of the mid-stream urine samples were collected after the patients had been fully instructed in the correct technique and after a complete vulval wash with soap. Aerobic and anaerobic cultures were undertaken on every sample and these were initiated within three hours of collection.

Clinical observations

Visit 1. At the first visit each patient had a blood sample taken under the basal conditions described. A detailed case history was then taken together with a full physical examination of all major systems including weight, blood pressure, pulse rate, breast palpation, a mid-stream specimen of urine (MSU) for aerobic and anaerobic culture, and cervical and vaginal cytology smears in duplicate. The duration of amenorrhoea was noted together with details of the number of flushing and sweating attacks occurring per day or per week. The patient's own assessment of her general energy and emotional status relative to the last five menstrual years was also noted.

Visit 2 (one week later). A blood sample only was obtained under the same basal conditions and the laboratory determinations detailed above were measured.

Visit 3 (a further week later). In addition to the blood sample a further MSU was obtained and the vaginal smears were repeated in duplicate.

The data from these three visits defined the pre-therapy status of each individual and the women were then randomized into one of seven possible therapy groups (detailed below). They were carefully instructed to take their tablets at breakfast time each day for 21 days and then to have seven days off therapy. Each package contained sufficient tablets for 21 days and a new supply was only given at the next visit. The appointments were arranged for day 21 of each cycle, namely the day the last pill was taken; the time that the pill was taken at home was noted by the patient on her own watch and the time the blood was obtained was also noted on the same watch. In this way, while the actual time of day might not have been accurate, the interval between pill ingestion and blood sampling was estimated with reasonable accuracy.

Visits 4 to 9. These were six consecutive monthly visits. As indicated above, the patients came on day 21 of each therapy cycle and had thus taken the last tablet of that particular course just before leaving home. The blood sample was always obtained under the same basal conditions and the pulse rate and blood pressure were determined in the rested state. Patients were weighed and an MSU obtained during visits 4, 5 and 6; if the urine was consistently sterile no further samples were taken. If infected and therapy prescribed, an MSU was taken at every visit. Further vaginal smears were obtained in duplicate during visits 4, 5, 6 and 9.

Breakfast was provided following these tests and the patients were then questioned about menstruation, hot flushes, any side-effects of therapy and their opinion with regard to their general energy and emotional status. After six months (visit 9) all therapy was stopped and the patient was asked to return three months later.

Visit 10. After three months off all therapy, every test and examination described was repeated.

The tests and observations made at each visit are summarized in Table II.

THERAPY GROUPS

At the time this study began there were four major forms of hormone replacement therapy: conjugated equine oestrogens (Premarin, 1.25 mg, *Ayerst*); piperazine oestrone sulphate (Harmogen, 1.5 mg, *Abbott*); oestradiol valerate (Progynova, 2 mg, *Schering*) and oestradiol valerate plus norgestrel, 0.5 mg, (Cycloprogynova, *Schering*). Only one firm (Schering) was producing a commercially available combined pill with an oestrone and progesterone effect but, at the suggestion of the firms producing Harmogen (Abbott) and Premarin (Ayerst), norethisterone acetate, 5 mg, was added to their products making a total of seven possible therapy groups: placebo; conjugated equine oestrogens (EO), 1.25 mg; EO, 1.25 mg, plus norethisterone acetate (NA), 5 mg; piperazine oestrone sulphate (E_1S), 1.5 mg; E_1S, 1.5 mg, plus NA, 5 mg; oestradiol valerate (E_2) 2 mg; and E_2, 2mg, plus norgestrel (Ng), 0.5 mg.

CLINICAL FINDINGS

In Table IV, the patients have been divided according to the duration of amenorrhoea, type of therapy prescribed and whether they completed all 10 test visits. Of the nine patients having amenorrhoea for less than 12 completed months, the durations were 6, 7, 9, 9, 10, 10, 11, 11 and 11 months respectively.

Seven patients withdrew before completing the whole trial. Their data are given below.

Patient 1. Amenorrhoea had been present for 12 months; for the first two months on EO a vaginal blood loss occurred in the seven days off therapy. These losses were described as being like 'normal periods'. Factor VIII activity was reported as 50 per cent of normal at the end of the second course of tablets. During the third month, irregular vaginal losses occurred while on therapy and Factor VIII was reported as only 25 per cent of normal. The loss in the seven days off therapy was described as 'flooding'. The patient remained off therapy and after four weeks, Factor VIII activity had returned to 70 per cent. Due to a misunderstanding the patient then re-started EO and 'flooding' began to occur within a few days. Repeated determinations of Factor VIII activity were always in excess of 80 per cent of the normal value but all therapy was discontinued. All of the other measured indices of clotting function had remained normal on all occasions. Dilatation and curettage of the uterus under general anaesthesia during the first month off therapy failed to obtain any endometrial curettings. The patient has subsequently remained asymptomatic and well but her hot flushes and sweating, relieved by therapy, have returned.

Patient 2. Amenorrhoea had been present for 30 months. After the first month of treatment (EO) her menopausal symptoms were improved but before starting the next course of tablets the patient rang to say 'her family wished her to discontinue the pills as she was so irritable and nervous'.

Patient 3. Amenorrhoea had been present for 10 months. During the first month on treatment (E_1S) her menopausal symptoms of hot flushes decreased but her 'migraine' headaches increased. During the third month, abdominal swelling was experienced and this symptom plus the headaches outweighed the benefits from the reduction in hot flushes and sweating. All symptoms subsided off therapy.

Patient 4. Amenorrhoea had been present for 24 months. During the first month on treatment (E_1S+NA) the patient felt unwell with indigestion. flatulance, and a general feeling of tiredness and depression. During the second month she stated that her husband found her irritable and aggressive and wished her to discontinue therapy. At follow-up she claimed to feel much better off therapy.

Patient 5. Amenorrhoea had been present for nine months. During the first month of therapy (E_2) there was complete freedom from hot flushes. No bleeding occurred during the seven days off therapy but during the first week of the second course of tablets a 'torrential' vaginal bleed occurred and lasted for one week. After therapy was stopped the bleeding continued and the patient was admitted to hospital. No curettings could be obtained with formal curettage under general anaesthesia but the bleeding gradually ceased over the following two days. At follow-up three months later, the patient had not had any further bleeding.

Patient 6. Amenorrhoea had been present for 60 months. This patient had never had hot flushes or sweating attacks but wished to take part in the trial to see if it would help her tiredness and mild depression. Menses were induced by therapy (E_2+Ng) and as she did not experience any symptomatic improvement after two months on HRT, she decided to withdraw from the study.

Patient 7. Amenorrhoea had been present for 12 months. With therapy (E_2+Ng) regular vaginal bleeding occurred which the patient was prepared to accept but she also developed renal angle pain and frequency of micturition. She wished to maintain her part in the trial and continued with HRT for five months. Despite the

TABLE IV

Patients divided according to their duration of amenorrhoea, the type of therapy prescribed and whether or not they completed all 10 visits

Treatment	Duration of amenorrhoea (months)			No. of patients
	Under 12	12–17	18+	
EO				
Completed	1	2	2	6
Withdrew	–	1	1	2
EO + NA				
Completed	1	2	5	8
Withdrew	–	–	–	–
E_1S				
Completed	–	1	6	7
Withdrew	1	–	–	1
E_1S + NA				
Completed	1	–	6	7
Withdrew	–	–	1	1
E_2				
Completed	2	–	5	7
Withdrew	1	–	–	1
E_2 + Ng				
Completed	1	–	5	6
Withdrew	–	1	1	2
All HRT				
Completed	6	6	29	41
Withdrew	2	2	3	7
Placebo				
Completed	1	–	7	8
Withdrew	–	–	–	–
All patients				
Completed	7	6	36	49
Withdrew	2	2	3	7

EO, Conjugated equine oestrogen; NA, Norethisterone acetate; E_1S, Piperazine oestrone sulphate; E_2, Oestradiol valerate; Ng, Norgestrel; Placebo, Calcium lactate.

fact that MSUs cultured aerobically and anaerobically failed to grow organisms, she continued to experience dysuria and frequency. Pus cells were never found and cultures for *Mycobacterium tuberculosis* were initiated; results were negative and an IVP was reported as normal. After therapy was stopped, all symptoms referable to the genito-urinary system disappeared but her hot flushes and sweating returned.

The type and duration of therapy taken by these patients are given in Table V. In the results which follow only the data from the 49 patients who completed the trial will be given. However, with the exception of the one fall in Factor VIII described, the data from the seven omitted patients were similar to those from the other women in the same therapy groups during the equivalent visits; they would not have affected the overall conclusions in any way.

Blood pressure

There was a small but statistically significant fall in the mean systolic and diastolic blood pressure in the 41 women taking HRT. After therapy was completed, both the systolic and diastolic pressures returned to the pretherapy values (Table VI). The fall in systolic pressure occurred in 30 of the 41 treated women and the diastolic pressure fell in 31 of the 41 patients. The patients given a placebo had higher average diastolic pressures than the patients given HRT. The mean pretreatment levels were 79, 79, 83, 89, 100, 101, 126 and 136 mmHg for the eight patients in this group. While some individuals in the HRT group had similar values, they were too few to affect the mean values to the same degree. The patient with a diastolic pressure of 136 mm Hg had a systolic pressure of 235 mm Hg and was treated with methyldopa before being given HRT.

Pulse rate

Pulse rate also decreased by a small but significant amount in the patients given HRT; the rate increased again when HRT was discontinued. No change was noted in the patients taking placebo (Table VI).

Weight and weight for height

There was no convincing evidence of a change in either absolute weight or weight expressed as a percentage of ideal body weight for height.

Induced uterine bleeding

None of the women given a placebo bled on any occasion and women who did have 'menses' while using HRT invariably ceased having bleeds at the end of six months of therapy. The relation between the type of HRT taken and the induction of uterine bleeding was, however, variable. The women taking an oestrogen-only preparation rarely had bleeding induced; those completing the trial and who received E_1S and E_2 did not bleed at all and only 3 of the 6 patients receiving EO had bleeding. The pattern of bleeding can be seen from Table VII where the concept of a 'theoretical maximum number of menses' has been used. This is the number of patients in any group multiplied by six (the number of therapy-months during which a bleed could have been induced). Thus the six women receiving E_2+Ng would yield 36 'menstrual periods' as their theoretical maximum. Only five women actually had menses induced but they yielded 29 bleeds hence each achieved a period virtually every month for the six months on therapy.

A combined preparation, whatever the type, caused a higher incidence of induced bleeding; every women on EO+NA bled as did 6 out of 7 on E_1S+NA and 5 out of 6 on E_2+Ng. The actual number of bleeds caused fell below the theoretical maximum in each group (Table VII).

Vaginal cytology

The changes in vaginal cytology were difficult to interpret. While, in general, women on HRT showed a decrease in the percentage of basal cells and an increase in the proportion of cornified cells, this was not invariable. Sometimes the two slides made from the same vagina on the same occasion would yield different cytological scores. The impression gained was that, cytologically, the vaginal mucosa did not respond homogenously over its entire surface. It was equally obvious that any 'improvement' in a given woman on HRT was not necessarily progressive. Thus a 'better' cytological picture could be found after say three months on HRT than after five or six months. A few women showed no significant 'improvement' at all.

TABLE V

Details of seven patients who failed to complete all 10 visits

Patient	Therapy	Visits attended*	Reason for stopping therapy
1	EO	1–6 and 10	Heavy vaginal bleeding
2	EO	1–4 and 10	Irritable and nervous
3	E_1S	1–6 and 10	Headaches, abdominal swelling
4	E_1S + NA	1–5 and 10	Abdominal pain, depression
5	E_2	1–4 and 10	Heavy vaginal bleeding
6	E_2 + Ng	1–5	Abdominal pain, indigestion
7	E_2 + Ng	1–8 and 10	Tiredness, depression

* 10 – indicates the patient was seen three months after the *last month on therapy despite not completing the whole course.*

TABLE VI

Blood pressure and pulse rate (mean ± SD) for the 49 women during the three major phases of the study according to whether they received HRT or a placebo

	Pretreatment (A)	Treatment (B)	Post-treatment (C)	Paired t-test		
				A *v* B	B *v* C	A *v* C
41 women given HRT						
Systolic blood pressure (mmHg)	136 ± 17	129 ± 17	137 ± 21	0.001	0.001	NS
Diastolic blood pressure (mmHg)	84 ± 9	81 ± 8	86 ± 11	0.001	0.001	NS
Pulse rate (beats/minute)	79 ± 12	75 ± 10	78 ± 16	0.001	0.05	NS
8 women given placebo						
Systolic blood pressure (mmHg)	152 ± 36*	142 ± 29	138 ± 23	0.05	NS	0.05
Diastolic blood pressure (mmHg)	98 ± 22	95 ± 18	97 ± 16	NS	NS	NS
Pulse rate (beats/minute)	77 ± 15	75 ± 9	76 ± 10	NS	NS	NS

NS = Not significant.

* One patient was found to have essential hypertension with a systolic blood pressure of 235 mmHg before HRT and was given methyldopa.

TABLE VII

Incidence and pattern of uterine bleeding according to the type of therapy taken. (See text for details)

Therapy group	No. of patients	No. reporting menses*	Theoretical maximum number of menses†	Actual number of menses‡
Placebo	8	0	48	0
EO	6	3	33§	15
EO + NA	8	8	48	42
E_1S	7	0	42	0
E_1S + NA	7	6	42	28
E_2	7	0	42	0
E_2 + Ng	6	5	36	29

* No. of patients who had a bleed on some occasion.
† No. of patients in the whole group times 6 (months on therapy).
‡ Total number of recorded uterine bleeds per group.
§ On 3 occasions presence or absence of a bleed not reported.

While there was some correlation between the clinical assessment of the vaginal mucosal status and the cytological picture, there were several exceptions in which an apparently moist and healthy vagina displayed a very atrophic cytological picture. There was no obvious correlation between the plasma levels of oestrone and oestradiol achieved and the cytology.

Urine culture

Only four women in the whole series had positive cultures and these were found in the pretreatment samples in each case; the organisms had presumably been present for some time but all of the patients were asymptomatic. No chemotherapy was given for the first three months on HRT to see if replacement therapy alone might be helpful; this was not the case and antibiotics were prescribed according to the sensitivity of the organisms concerned.

Patient A. The MSU contained an average of 250 pus cells/µl and cultured *Escherichia coli.* An IVP was reported as normal and the patient responded to ampicillin.

Patient B. There were 33 pus cells/µl on average and *E. coli* was cultured. An IVP was normal and the patient responded to septrin.

Patient C. There were 10 pus cells/µl on average; *Streptococcus viridans* was cultured on one occasion and coagulase negative staphylococci on others. This patient withdrew from the trial before further investigations could be instigated.

Patient D. There were 30 pus cells/µl on average; bacteria were cultured in profusion and identified as lactobacilli on each occasion. These were assumed to be contaminants despite a careful collection technique but in view of their persistence and the presence of pus cells, a catheter specimen of urine was obtained with full aseptic technique and again lactobacilli were cultured. After a course of ampicillin the urine became sterile but two months later the organisms were cultured again. An IVP was normal and the patient appears to have a chronic tendency to carry these organisms in her bladder without these affecting her general health or causing symptoms.

Hot flushes

The degree to which women experienced discomfort from 'hot flushes' was extremely variable. Some had no such sensations at all, others experienced a feeling of flushing on two or three occasions per week without perspiring, while others could suffer six or more flushes per day and perspire profusely. The complaint which most obviously affected the quality of life was that of night perspiration. This could be so severe that some patients had to change their bed linen at least once each night.

Because of this variability we have simply recorded whether the patients experienced hot flushes at all and, if so, whether they improved or not with HRT. Those who did not have such sensations at the beginning of the trial could only continue to report 'none' or perhaps 'more' if attacks began while on HRT. The other patients were asked to grade the number of flushing attacks as none, fewer, the same or more, relative to their frequency before therapy. The same system was used to describe their status at the last visit three months after therapy had been discontinued. There were no obvious differences in response between the various HRT groups so the patients were grouped according to whether they had, or had not, experienced hot flushes before treatment and whether they received HRT or placebo. Of the 30 women receiving HRT and who had experienced flushing attacks, most obtained symptomatic relief. As each woman was questioned each month there were therefore 6×30 or 180 'women occasions' on which their flush status could be recorded. On only 12 of these occasions were hot flushes reported as 'the same' or 'worse' than before taking HRT. However, improvement also occurred on 22 of the 42 'women occasions' reported by the seven women taking a placebo who had previously had hot flushes (Table VIII). This was largely due to three women who reported an improvement on every occasion.

LABORATORY FINDINGS

In the following tables the pretreatment data are the mean of the three tests before HRT, the treatment values the mean of the six tests performed while on therapy and the post-treatment values are derived from the single test occasion three months after HRT had been dis-

TABLE VIII

*Incidence of 'hot flushes' on HRT or placebo. 'YES' and 'NO' refer to whether each patient did, or did not, experience 'flushes' prior to the trial**

Therapy group	Before therapy	Incidence of hot flushes							
		During therapy				Three months off therapy			
		None	Fewer	Same	More	None	Fewer	Same	More
All HRT	YES (n = 30)	124	45	8	3	6	–	4	20
	NO (n = 11)	65	–	–	1	10	–	–	1
Placebo	YES (n = 7)	6	16	16	4	–	1	4	2
	NO (n = 1)	6	–	–	–	1	–	–	–

* Each patient made a response during each of the six therapy months and once when off therapy for three months (e.g. 30 patients would yield 6 × 30 possible responses on therapy = 180 and 30 × 1 responses off therapy).

continued. Mean values and standard deviations are given for each therapy group in the tables but the number of patients in the individual groups is too small for reliable analysis. The patients who received HRT are therefore combined as one group for the three occasions 'pretreatment', 'treatment' and 'post-treatment' for statistical analysis. The placebo group is shown separately.

FSH and LH

The levels of FSH decreased significantly in women receiving HRT, the values returning to the pretreatment concentrations after therapy had been discontinued (Table IX). The same was true for LH (Table X). The decrease in FSH on treatment was shown by 40 of the 41 women and in LH concentration by 36 of the 40 women.

It appears that the combined preparations caused a greater decrease in gonadotrophin levels than the oestrogen-only agents but the numbers in each individual therapy group were too small for statistical testing. However, combining the data from the women taking oestrogens only into one group and comparing them with the data from the women on an oestrogen-progestogen preparation as a single group revealed that the decrease in serum concentration of both FSH and LH was significantly greater in the women taking the combined preparations (Table XI).

The ratio of FSH/LH changed during the time HRT was taken from 1.14 (± 0.34) to 0.96 (± 0.28) due to a proportionally greater suppression of FSH (Table XII).

Oestrone

Of the 8 patients randomized into the group

TABLE IX

Serum FSH levels (Mean ± SD) for each treatment group before, during and after HRT or placebo

Therapy group	Serum FSH levels (U/l)			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	66 ± 19 (6)	38 ± 4 (6)	77 ± 30 (5)	–	–	–
EO + NA	66 ± 18 (8)	28 ± 14 (8)	79 ± 25 (7)	–	–	–
E_1S	72 ± 20 (7)	58 ± 14 (7)	85 ± 20 (7)	–	–	–
E_1S + NA	65 ± 15 (7)	39 ± 9 (7)	69 ± 14 (7)	–	–	–
E_2	78 ± 14 (7)	55 ± 15 (7)	77 ± 15 (6)	–	–	–
E_2 + Ng	69 ± 31 (6)	39 ± 17 (6)	77 ± 27 (6)	–	–	–
All HRT	69 ± 19 (41)	43 ± 24 (41)	77 ± 21 (38)	0.001	0.001	NS
Placebo	85 ± 22 (8)	83 ± 24 (8)	82 ± 26 (8)	NS	NS	NS

Number of patients in parentheses.

TABLE X

Serum LH levels (Mean ± SD) for each treatment group before, during and after HRT or placebo

Therapy group	Serum LH levels (U/l)			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	70 ± 25 (6)	55 ± 18 (6)	55 ± 14 (5)	–	–	–
EO + NA	67 ± 15 (7)	34 ± 15 (8)	77 ± 20 (7)	–	–	–
E_1S	60 ± 11 (7)	55 ± 10 (7)	60 ± 15 (7)	–	–	–
E_1S + NA	58 ± 16 (7)	41 ± 9 (7)	61 ± 10 (7)	–	–	–
E_2	73 ± 17 (7)	65 ± 12 (7)	70 ± 22 (7)	–	–	–
E_2 + Ng	72 ± 40 (6)	48 ± 23 (6)	58 ± 32 (6)	–	–	–
All HRT	67 ± 21 (40)	49 ± 17 (41)	64 ± 20 (39)	0.001	0.001	NS
Placebo	56 ± 8 (8)	52 ± 07 (8)	55 ± 9 (8)	NS	NS	NS

Number of patients in parentheses.

TABLE XI

Decrement in serum levels of FSH and LH (pretreatment value minus *treatment value) according to whether the women received oestrogens only or an oestrogen-progestogen preparation*

	FSH (U/l)		LH (U/l)	
	Oestrogens only	Oestrogen-progestogen	Oestrogens only	Oestrogen-progestogen
Number	20	21	20	20
Decrement				
Mean	21.3	31.5	9.3	24.7
SD	16.8	14.2	13.7	17.2
	* $p < 0.05$		* $p < 0.01$	

* Significance of difference between the two therapy groups.

TABLE XII

Ratio of serum FSH to LH levels for each treatment group before, during and after HRT or placebo

Therapy group	Ratio of serum FSH to LH levels			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	1.01 ± 0.37 (6)	0.79 ± 0.28 (6)	1.46 ± 0.72 (5)	–	–	–
EO + NA	1.11 ± 0.28 (7)	0.89 ± 0.14 (8)	1.02 ± 0.20 (7)	–	–	–
E_1S	1.25 ± 0.37 (7)	1.08 ± 0.16 (7)	1.47 ± 0.44 (7)	–	–	–
E_1S + NA	1.20 ± 0.39 (7)	1.12 ± 0.18 (7)	1.18 ± 0.42 (7)	–	–	–
E_2	1.13 ± 0.29 (7)	0.93 ± 0.27 (7)	1.34 ± 0.67 (6)	–	–	–
E_2 + Ng	1.09 ± 0.44 (6)	0.96 ± 0.50 (6)	1.42 ± 0.44 (6)	–	–	–
All HRT	1.14 ± 0.34 (40)	0.96 ± 0.28 (41)	1.30 ± 0.49 (38)	0.001	0.001	NS
Placebo	1.57 ± 0.47 (8)	1.70 ± 0.50 (8)	1.51 ± 0.45 (8)	NS	NS	NS

Number of patients in parentheses.

taking placebo, 7 had had amenorrhoea for at least 18 months. Their mean endogenous oestrone values were low with a small standard deviation over the 10 months of the trial and probably represent the true state when spontaneous ovarian function has ceased (Table XIII). While the mean pre-treatment values were about the same in the HRT groups there were some individuals who had a wide range of values, indicating that in a few patients endogenous ovarian function was still occurring. One woman allocated to the group to be given oestrone sulphate had pretreatment serum oestrone levels of 491, 767 and 255 pm/l respectively over the three weeks before therapy, presumably indicating spontaneous follicular activity. The effect on the standard deviation can be seen in Table XIII. By the time of the last test, three months after all therapy had been discontinued, the endogenous levels were again low but now the standard deviations for each group were equivalent to those found in the placebo group.

The standard deviations of serum oestrone values were very large in each group while taking HRT. This will be commented upon below. However, all 41 patients receiving HRT showed a rise in serum oestrone levels relative to their own pretreatment values.

Oestradiol

The same comments on oestrone are also true for oestradiol. The patients taking placebo had a low average value but this was not the case for the pretreatment concentrations in several of the HRT groups. In the patient referred to above, endogenous oestradiol values for the three weeks before therapy were 1138, 1742 and 465 pmol/l

TABLE XIII

Serum oestrone levels (Mean ± SD) for each treatment group before, during and after HRT or placebo

Therapy group	Serum oestrone levels (pmol/l)			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	170 ± 86 (6)	554 ± 78 (6)	112 ± 30 (5)	–	–	–
EO + NA	155 ± 66 (8)	522 ± 222 (8)	152 ± 43 (7)	–	–	–
E_1S	164 ± 156 (7)	755 ± 271 (7)	104 ± 41 (7)	–	–	–
E_1S + NA	131 ± 47 (7)	527 ± 206 (7)	111 ± 32 (7)	–	–	–
E_2	95 ± 18 (7)	944 ± 597 (7)	135 ± 30 (7)	–	–	–
E_2 + Ng	122 ± 12 (6)	533 ± 169 (6)	94 ± 31 (5)	–	–	–
All HRT	140 ± 80 (41)	641 ± 332 (41)	120 ± 39 (38)	0.001	0.001	NS
Placebo	127 ± 37 (8)	146 ± 38 (8)	144 ± 33 (7)	NS	NS	NS

Number of patients in parentheses

TABLE XIV

Serum oestradiol levels (Mean ± SD) for each treatment group before, during and after HRT or placebo

Therapy group	Serum oestradiol levels (pmol/l)			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	184 ± 146 (6)	332 ± 72 (6)	71 ± 44 (5)	–	–	–
EO + NA	130 ± 124 (8)	290 ± 103 (8)	178 ± 258 (7)	–	–	–
E_1S	224 ± 393 (7)	246 ± 48 (7)	93 ± 70 (7)	–	–	–
E_1S + NA	156 ± 184 (7)	221 ± 76 (7)	78 ± 45 (7)	–	–	–
E_2	83 ± 18 (7)	367 ± 151 (7)	54 ± 13 (7)	–	–	–
E_2 + Ng	98 ± 51 (6)	255 ± 117 (6)	69 ± 18 (6)	–	–	–
All HRT	146 ± 191 (41)	285 ± 107 (41)	92 ± 117 (39)	0.001	0.001	NS
Placebo	88 ± 35 (8)	85 ± 30 (8)	99 ± 63 (8)	NS	NS	NS

Number of patients in parentheses.

respectively. This endogenous activity appears to have ended by the time the last test was performed in all but the group receiving EO+NA (Table XIV). The standard deviations of oestradiol values for all of the groups receiving HRT were quite large and this will be discussed in more detail later; however, 38 of the 41 women showed an increase in serum oestradiol concentration while on therapy relative to her own average pretreatment value.

As with FSH and LH concentrations, it appears that the average serum concentrations of both oestrone and oestradiol achieved when a *combined* oestrogen and progestogen is taken are somewhat lower than when an oestrogen alone is taken, but it must be emphasized that the pre-therapy serum concentrations are too variable to test the reality of this observation.

Ratio of oestradiol to oestrone

Irrespective of whether the hormonal preparation contained conjugated equine oestrogens, piperazine oestrone sulphate or oestradiol valerate, the effect was to increase the level of circulating *oestrone* more than *oestradiol*. This can be seen in Table XV where the ratio of E_2/E_1 decreased in each therapy group other than those receiving the placebo; this was always caused by the proportionately greater rise in serum oestrone concentration.

Progesterone

None of the patients had significantly different

TABLE XV

Ratio of serum oestradiol to oestrone levels for each treatment group before, during and after HRT or placebo

Therapy group	Ratio of serum oestradiol to oestrone levels			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	0.97 ± 0.29 (6)	0.64 ± 0.25 (6)	0.66 ± 0.38 (5)	–	–	–
EO + NA	0.75 ± 0.34 (8)	0.69 ± 0.20 (8)	1.17 ± 1.60 (7)	–	–	–
E_1S	0.98 ± 0.57 (7)	0.37 ± 0.12 (7)	1.04 ± 0.79 (7)	–	–	–
E_1S + NA	1.06 ± 0.68 (7)	0.48 ± 0.23 (7)	0.74 ± 0.40 (7)	–	–	–
E_2	0.96 ± 0.27 (7)	0.45 ± 0.12 (7)	0.41 ± 0.12 (7)	–	–	–
E_2 + Ng	0.77 ± 0.27 (6)	0.54 ± 0.23 (6)	0.75 ± 0.34 (5)	–	–	–
All HRT	0.91 ± 0.43 (41)	0.53 ± 0.22 (41)	0.80 ± 0.80 (38)	0.001	0.05	NS
Placebo	0.71 ± 0.18 (8)	0.61 ± 0.21 (8)	0.69 ± 0.39 (7)	NS	NS	NS

Number of patients in parentheses.

TABLE XVI

Serum progesterone levels (Mean ± SD) for the women receiving HRT or placebo before, during, and after therapy together with serum norethisterone and norgestrel levels (Mean ± SD) for those women receiving these particular preparations

Therapy group	Serum progestogen	Period of sampling		
		Pretreatment	During treatment	Post-treatment
All HRT	Progesterone (pmol/l)	124 ± 99 (41)	108 ± 71 (41)	89 ± 33 (39)
Placebo	,,	126 ± 44 (8)	109 ± 41 (8)	104 ± 44 (8)
EO + NA	Norethisterone (nmol/l)	–	61.8 ± 21.2 (8)	–
E_1S + NA	,,	–	35.2 ± 11.9 (7)	–
E_2 + Ng	Norgestrel (nmol/l)	–	13.7 ± 5.3 (6)	–

Number of patients in parentheses.

levels of endogenous progesterone before, during or after therapy. Thus the few patients with high endogenous values for oestrone and oestradiol must have had residual follicular activity but not ovulation. The patient quoted above had, during the three pretreatment weeks, oestradiol values of 1138, 1742 and 465 pmol/1, and her progesterone concentrations were only 100, 100 and 140 pg/ml respectively. The mean values for all the HRT groups and the placebo group are given in Table XVI.

Norethisterone and norgestrel

The average serum concentrations achieved in those women receiving these preparations are given in Table XVI. The levels of norethisterone achieved in conjunction with conjugated equine oestrogens were significantly greater than those achieved with piperazine oestrone sulphate ($p<0.01$).

Prolactin

Prolactin concentrations were measured in nine women deliberately chosen because they achieved serum levels of oestrone and oestradiol with HRT equivalent to those expected during a normal menstrual cycle. There was no significant change: the mean pretreatment serum prolactin concentration was 165 mU/1, and after HRT it was 167 mU/1. These findings have been discussed in detail elsewhere (Lind et al, 1978).

Hormone concentrations relative to the interval between tablet ingestion and blood sampling

As indicated previously, the interval between the patient taking the tablet and the blood sample being obtained was carefully noted for subsequent linking to the estimates of serum oestrone, oestradiol and, where appropriate, serum norethisterone and norgestrel. However, the patients had already been taking HRT for 20 days before the blood samples were obtained for analysis, and nothing was known about the hormone concentrations which would be achieved or the effect of the time interval between the taking of the tablet(s) and the blood sample being obtained. In an attempt to clarify the sequence of events following tablet ingestion, 10 patients were asked to volunteer for a further test procedure when they were completing their third month on a second course of HRT. (Patients wishing to take a subsequent course of HRT are discussed in detail later). Each attended the Unit on the morning the last pill of this third cycle was to be taken. Each had again fasted overnight but the tablet was *not* taken at home. After the patient had been sitting quietly at rest in a comfortable chair, a blood sample was obtained by venepuncture in the usual way (zero time) and the patient then swallowed the tablet(s). Further blood samples were obtained at 15, 30, 45, 60, 90 and 120 minutes afterwards. Oestrone and oestradiol were assayed on every sample and, where appropriate, norethisterone and norgestrel. The data are given in Table XVII.

In three patients (Patients 1, 4 and 5) there

TABLE XVII

Serum oestrone (E_1), oestradiol (E_2) and, where appropriate, norethisterone (Nor) and norgestrel (Ng) levels over two hours in 10 patients receiving various forms of HRT. The values at time zero are those obtained before the tablet(s) were swallowed. Each test was performed on day 21 of the therapy cycle

Patient	Therapy taken	Serum hormone	Time (minutes)						
			0	15	30	45	60	90	120
1	EO	E_1 (pmol/l)	329	[illegible]	497	866	1015	1271	1269
		E_2 (pmol/l)	130	[illegible]	412	729	635	354	345
2	EO	E_1 (pmol/l)	338	323	309	312	295	308	317
		E_2 (pmol/l)	225	[illegible]	207	209	207	230	228
3	EO	E_1 (pmol/l)	544	458	364	473	466	367	476
		E_2 (pmol/l)	275	[illegible]	292	256	261	273	257
4	EO	E_1 (pmol/l)	554	548	894	1196	1333	1552	1638
		E_2 (pmol/l)	215	[illegible]	703	626	*	*	435
5	E_2	E_1 (pmol/l)	466	983	1004	1069	1232	1306	*
		E_2 (pmol/l)	276	[illegible]	835	736	1395	560	511
6	EO + NA	E_1 (pmol/l)	744	[illegible]	419	759	732	711	748
		E_2 (pmol/l)	370	[illegible]	364	398	314	311	257
		Nor†	3.3	3.3	3.3	3.3	3.3	3.3	3.3
7	EO + NA	E_1 (pmol/l)	882	*	923	812	943	1068	1241
		E_2 (pmol/l)	283	[illegible]	260	281	292	280	281
		Nor†	3.3	3.3	3.3	3.3	3.3	3.3	3.3
8	E_1 S + NA	E_1 (pmol/l)	508	[illegible]	*	551	522	562	638
		E_2 (pmol/l)	238	[illegible]	263	218	251	233	223
		Nor†	3.3	3.3	3.3	3.3	3.3	3.3	3.3
9	E_1 S + NA	E_1 (pmol/l)	756	[illegible]	751	682	745	768	829
		E_2 (pmol/l)	314	[illegible]	296	269	289	255	302
		Nor (nmol/l)	13.7	[illegible]	29.9	47.9	47.8	56.6	49.4
10	E_2 + Ng	E_1 (pmol/l)	*	[illegible]	*	668	805	*	994
		E_2 (pmol/l)	240	[illegible]	338	378	338	318	333
		Ng‡	0.45	0.46	0.45	0.46	0.46	0.46	0.46

* Assay duplication poor and insufficient serum for repeat.
† All values *less than* 3.3 nmol/l.
‡ All values *less than* 0.46 nmol/l.

appeared to be evidence of absorption within the two hours as evidenced by the rising concentrations of both oestrone and oestradiol. In a fourth patient (Patient 7) there was a rise in oestrone concentration after 90 minutes but this did not reflect in the oestradiol or norethisterone levels. In 5 of the remaining 6 patients, there was no convincing evidence of a rise in concentration of any of the hormones while in the sixth (Patient 9) there was a convincing increase in serum norethisterone levels unaccompanied by any change in E_1 or E_2 values. *The major finding was that in each of the 10 patients the oestrone concentrations at zero time, namely about 24 hours after the last tablet had been taken, were still well above the average pretreatment levels.*

Analysis of the serum hormone concentrations at the stated time intervals since the tablets were taken in the 41 patients over the six months on therapy showed so much fluctuation both within individuals from occasion to occasion and across individuals that no relation between time and concentration could be demonstrated. The hormonal data from three patients taking different preparations during each of the six months on therapy are given in Table XVIII as examples. Not only is there no relation between the time the tablets were taken and the serum concentrations of the hormones measured but there is no apparent consistency in the relations between the hormones themselves. The data in Table XVIII have been arranged according to the time interval between tablet ingestion and blood sampling and not by the months on therapy. In Patient A the total time interval spanned is only 15 minutes yet there is a wide range of values of all the hormones measured. In Patient B the highest oestrone value (63 minutes) is associated with a relatively low oestradiol value but the highest norethisterone concentration. However, essentially the same oestradiol concentration is associated with the lowest norethisterone value at 52 minutes. Patient C shows a wide range of oestradiol and norgestrel values for essentially the same oestrone concentrations at 75 and 81 minutes.

TABLE XVIII

Serum hormone levels for three patients (as examples) arranged in order of increasing time interval from the time the tablets were taken and the blood obtained on six occasions (not in date order)

Serum hormone	Time intervals, arranged sequentially, between tablet ingestion and blood sampling (minutes)					
			Patient A			
	15	*17*	*25*	*26*	*27*	*30*
E_1 (pmol/l)	450	888	451	536	654	84
E_2 (pmol/l)	226	358	159	257	215	326
Nor (nmol/l)	83	66	91	52	53	102
			Patient B			
	41	*52*	*60*	*63*	*72*	*72*
E_1 (pmol/l)	326	346	301	425	362	289
E_2 (pmol/l)	85	143	184	145	230	136
Nor (nmol/l)	34	19	49	76	30	45
			Patient C			
	45	*55*	*75*	*81*	*95*	*95*
E_1 (pmol/l)	507	608	978	920	696	528
E_2 (pmol/l)	134	237	255	535	296	861
Ng (nmol/l)	14	10	9	19	12	11

Nor = norethisterone.
Ng = norgestrel.
E_1 = oestrone.
E_2 = oestradiol.

Serum cholesterol

There was a small but statistically significant decrease in cholesterol in the 41 women taking HRT when analysed as a single group (Table XIX). However, in the 20 women taking an oestrogen-only compound, the average decrease was 0.35 mmol/l while in the 21 women receiving a combined preparation the average decrease was 1.00 mmol/l. This difference is significant ($p<0.01$), 14 out of the 20 women having a decreased concentration in the oestrogen-only group and 19 out of 21 in the other.

Serum triglyceride

There was no significant change in triglyceride concentration when the patients on HRT were taken as one group (Table XX). When they were again divided into the two therapy groups described above, it was found that for the 21 receiving combined therapy, 16 demonstrated a decrease, with an average reduction for the group of 0.24 mmol/l; only 8 of the 20 women receiving oestrogens alone showed a decrease in triglyceride concentration and the average for the group was a *rise* of 0.07 mmol/l. The difference between the two therapy groups was again significant ($p<0.01$).

Fasting plasma glucose

There was no convincing change in this value during therapy. The mean pretreatment value was 5.2±0.5 mmol/l and the treatment value 5.1±0.4 mmol/l in the 41 patients receiving HRT.

Serum urea and creatinine

There was a statistically significant decrease in mean urea concentration between the pretreatment value and treatment value in the group as a whole who received HRT; this decrease reversed after therapy was completed. However, this fall was less convincing when individual changes were examined (Table XXI). There was no appreciable change in the creatinine concentrations.

Haematological indices

Haemoglobin. There was a small but statistically significant decrease in haemoglobin concentration in the treated group from an average pretreatment value of 13.95 g/dl to 13.76 ($p<0.01$); this fall occurred in 29 of the 41 patients. There was a similar change in the red cell count the decrease occurring in 30 of the 41 patients. These and the following data are shown in Table XXII.

MCV. There was no significant change in mean cell volume in any of the patients; the mean treatment values in the placebo group and HRT group were 85.0 fl and 86.8 fl respectively.

Haematocrit. Despite the MCV remaining stable, the change in red cell count was sufficient to affect the haematocrit in patients on HRT. The mean pretreatment value was 40.84 l/l and 40.35 l/l on HRT which achieves significance in this group of 41 patients ($p<0.01$). There was no significant difference in any of these parameters according to whether oestrogens alone or in combination with a progestogen were taken.

TABLE XIX

Serum cholesterol levels for each treatment group before, during and after HRT or placebo

Therapy group	Serum cholesterol levels (mmol/l)			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	6.36 ± 1.04 (6)	5.81 ± 0.71 (6)	6.59 ± 1.23 (5)	–	–	–
EO + NA	6.58 ± 0.99 (8)	5.40 ± 0.76 (8)	6.17 ± 1.04 (8)	–	–	–
E_1S	6.54 ± 1.13 (7)	6.58 ± 0.85 (7)	7.25 ± 1.01 (7)	–	–	–
E_1S + NA	6.95 ± 1.05 (7)	6.33 ± 0.85 (7)	6.14 ± 0.88 (7)	–	–	–
E_2	7.45 ± 1.66 (7)	6.89 ± 1.15 (7)	7.41 ± 1.52 (7)	–	–	–
E_2 + Ng	6.72 ± 0.68 (6)	5.52 ± 1.18 (6)	5.84 ± 1.05 (6)	–	–	–
All HRT	6.77 ± 1.12 (41)	6.09 ± 1.03 (41)	6.58 ± 1.22 (40)	0.001	0.001	NS
Placebo	6.67 ± 1.07 (8)	6.44 ± 1.12 (8)	6.93 ± 1.16 (8)	NS	0.01	NS

Number of patients in parentheses.

TABLE XX

Serum triglyceride levels for each treatment group before, during and after HRT or placebo

Therapy group	Serum triglyceride levels (mmol/l)			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	1.11 ± 0.34 (6)	1.17 ± 0.29 (6)	1.08 ± 0.50 (5)	–	–	–
EO + NA	1.28 ± 0.52 (8)	1.08 ± 0.30 (8)	1.06 ± 0.32 (8)	–	–	–
E_1S	1.22 ± 0.42 (7)	1.20 ± 0.21 (7)	1.19 ± 0.29 (7)	–	–	–
E_1S + NA	1.28 ± 0.34 (7)	1.14 ± 0.39 (7)	1.16 ± 0.70 (7)	–	–	–
E_2	1.02 ± 0.30 (7)	1.19 ± 0.28 (7)	1.30 ± 0.57 (7)	–	–	–
E_2 + Ng	1.25 ± 0.36 (6)	0.85 ± 0.22 (6)	0.97 ± 0.42 (6)	–	–	–
All HRT	1.20 ± 0.38 (41)	1.11 ± 0.29 (41)	1.13 ± 0.47 (40)	NS	NS	NS
Placebo	0.97 ± 0.13 (8)	1.04 ± 0.31 (8)	1.22 ± 0.62 (8)	NS	NS	NS

Number of patients in parentheses.

TABLE XXI

Serum urea levels for each treatment group before, during and after HRT or placebo

Therapy group	Serum urea levels (mmol/l)			Paired t-test		
	Pretreatment (A)	Treatment (B)	Post-treatment (C)	A *v* B	B *v* C	A *v* C
EO	4.72 ± 1.07 (6)	4.14 ± 1.11 (6)	4.60 ± 1.14 (5)	–	–	–
EO + NA	3.84 ± 0.68 (8)	3.46 ± 0.80 (8)	4.11 ± 1.17 (8)	–	–	–
E_1S	4.07 ± 0.71 (7)	4.29 ± 0.67 (7)	4.08 ± 0.73 (7)	–	–	–
E_1S + NA	4.17 ± 0.82 (7)	4.10 ± 0.87 (7)	4.30 ± 0.38 (7)	–	–	–
E_2	4.38 ± 0.74 (7)	4.38 ± 0.73 (7)	4.85 ± 0.66 (7)	–	–	–
E_2 + Ng	4.00 ± 0.68 (6)	3.53 ± 0.46 (6)	3.93 ± 0.73 (6)	–	–	–
All HRT	4.18 ± 0.79 (41)	3.98 ± 0.83 (41)	4.30 ± 0.85 (40)	0.05	0.05	NS
Placebo	4.33 ± 0.83 (8)	4.42 ± 0.97 (8)	4.86 ± 1.49 (8)	NS	NS	0.05

Number of patients in parentheses.

TABLE XXII

Mean values for various haematological factors in the HRT group (41 patients) and placebo group (8 patients) before, and during, therapy

Haematological indices	Pretreatment value		Treatment value	
	All HRT	Placebo	All HRT	Placebo
Haemaglobin (g/dl)	13.95 ± 1.0	13.88 ± 1.2	13.76 ± 1.2	13.83 ± 1.0
Mean cell volume (fl)	86.5	85.1	86.8	85.0
Haematocrit (l/l)	40.84 ± 3.1	40.35 ± 2.7	40.35 ± 2.8	40.4 ± 2.8
Fibrinogen (g/l)	2.54 ± 0.58	2.26 ± 0.53	2.42 ± 0.35	2.48 ± 0.38
FDP (μg/ml)	< 10	< 10	< 10	< 10
Factors (per cent of control) V	92 ± 18	92 ± 25	98 ± 19	94 ± 12
VII	107 ± 16	99 ± 21	102 ± 14	121 ± 14
VIII	105 ± 33	80 ± 19	107 ± 23	117 ± 15
X	106 ± 16	103 ± 15	109 ± 17	115 ± 16
Prothrombin time (per cent of control)	98 ± 9	97 ± 6	96 ± 5	99 ± 4
PTTK (per cent of control)	109 ± 14	110 ± 12	107 ± 9	108 ± 8
Platelet count (10^9/l)	273 ± 53	270 ± 58	275 ± 43	269 ± 40

FDP = fibrogen degradation products.
PTTK = partial thromoplastin time with kaolin.

Clotting factors

Fibrinogen and FDP. No changes occurred throughout therapy in either the placebo or treated groups (Table XXII).

Factors V, VII, VIII and X. No significant changes occurred in the percentages of these factors relative to normal control plasma in any group (Table XXII).

Prothrombin time and PTTK. These remained unchanged in all groups (Table XXII).

Platelet count, ADP and collagen aggregation. These too were unchanged by HRT (Table XXII).

PRELIMINARY CONCLUSIONS

Initial patient response

In recent years, articles and television programmes aimed at women in the menopausal years have tended to give the impression that most women wish to benefit from HRT and the major barrier to its widespread use is the reluctance of the medical profession to prescribe it. It is interesting to note therefore that in the whole of our series only 59 per cent of the patients approached bothered to reply to the questionnaire. It could be argued that the remaining 41 per cent wished to avail themselves of the therapy but could not, or would not, help in the research. However, a prepaid envelope had been enclosed so that answers to a few simple questions could be returned and this was not used. It was also obvious that the general practitioners who were cooperating were at least willing to discuss problems of the menopause and their management but they did not receive more requests for HRT during normal surgery hours after our letters were sent out. Our women were from an industrial area which is economically depressed and conclusions drawn from their response may not be representative of the country as a whole. However, the patients approached included women in the professions, shop owners and business women, as well as women who cleaned floors as a full-time occupation. Some 45 per cent were home owners and 55 per cent lived in rented accommodation.

It is of interest to note the pattern of response in the 294 women who did reply. Of the 121 still menstruating and who could not, therefore, take part in our survey, 93 (77 per cent) would have volunteered. Amongst the 173 who were postmenopausal, only 115 (66 per cent) agreed to help. It seems possible that this reduction reflects the fact that women who have not undergone the menopause assume it is going to be a difficult time; but once the menopause has occurred many women may not be particularly troubled and have little reason to seek medical aid.

Blood pressure and pulse rate

It could be argued that the fall in systolic and diastolic blood pressure and pulse rate which occurred in women given HRT was due to a spurious increase in the pretreatment levels due to the nervousness of the patients at their initial visit. However, the pretreatment readings were the mean of six observations over three weeks, namely, duplicate readings at each visit. The values also returned to their original levels after therapy was discontinued.

While the reductions in both blood pressure and pulse rate are statistically significant, they are relatively small in clinical terms; nevertheless, it is interesting that the effect is opposite to the hypertensive effect noted in some women taking oral contraceptive agents. It seems unlikely that this hypotensive action will be useful in the control of essential hypertension but HRT can be safely prescribed for women with this condition who require symptomatic relief of postmenopausal flushes.

Induced uterine bleeding

Of the 41 treated women completing the trial, 11 did not experience flushing attacks before the trial and could not therefore be 'improved' in that sense. In 7 of these 11 women, uterine bleeding was induced and was totally unacceptable to them. In the remaining 30 women, symptomatic improvement was reported in the great majority of patients and bleeding was accepted as the 'price to be paid' for this improvement. However, when the question "Would you prefer to have your improvement without periods" was posed they invariably replied in the affirmative. Those who had relief from flushes but did not bleed were more hesitant when asked "Would your present improvement be sufficient to make you accept regular bleeds as a necessary part of your therapy". They had no basis for comparison of course, as they had no idea whether such 'periods' would be heavy or light, painful or painfree. The distinct clinical impression was gained that all of the women would prefer to have the symptomatic relief without the bleeding but that symptomatic improvement was usually such that bleeding would be endured if there were no alternative.

On the combined preparations, bleeding did not always begin after the first course of tablets but, once established, was regular thereafter. The loss was usually described as 'lighter' than the

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average menstrual loss over the last five years of their menstrual career, but occasionally was said to be 'the same' and rarely 'heavier' than the previous menstrual periods of any given individual. It should be remembered that two patients, described in detail earlier, did not complete the trial because of heavy vaginal bleeding.

Of the oestrogen-only compounds, conjugated equine oestrogens (Premarin) induced the greatest incidence of uterine bleeding; of the combined preparations it was again conjugated equine oestrogens which induced bleeding on the greatest number of occasions.

Two patients did not bleed on *any* occasion despite receiving a combined preparation: one received E_1S+NA and the other E_2+Ng. Of the remaining six women receiving E_1S+NA, five did not menstruate after the first course and three did not bleed after the second course either. All of the women reported their subsequent bleeds as 'lighter' than normal menses. The same applied, though to a lesser extent, for the women receiving E_2+Ng.

If the induction of regular uterine bleeding is thought to be protective against possible endometrial carcinoma, more work into the formulation of combined preparations is going to have to be undertaken to achieve a mixture which will induce convincing uterine bleeding in *all* patients on *every* occasion. The corollary could be that some women will bleed heavily on such preparations and patient acceptability will be correspondingly lower.

Hot flushes

Attacks of flushing were the most disagreeable aspect of the menopause for most of the women interviewed. Most agreed that they did not flush in the sense that their facial colour heightened or that other people were aware of their feeling but some did experience embarrassing perspiration with the flushes. In a minority of women, the perspiration was sufficient to soak through to their dress during the day or to require a change of bed linen during the night. In one instance, a husband had begun to sleep in a separate bed because he found the profuse sweating of his wife so unpleasant. In the majority of women, either complete relief from these symptoms was experienced while on HRT or the symptoms were considerably reduced in incidence and severity. All of the women having perspiration attacks experienced complete cessation of this symptom.

Our data do not throw light on the cause of such symptoms. Most of the women taking a placebo also experienced some lessening of symptoms. The endogenous levels of oestrogens before therapy did not relate to the symptoms and the induced oestrogen and progestogen levels did not relate to the abatement of symptoms. Thus the patient with the highest recorded levels of oestrone and oestradiol before HRT was experiencing hot flushes and night perspiration though not as severe as some patients. Other women with low endogenous oestrogen levels had no symptoms at all.

FSH and LH

As expected, the 49 patients taking part in the trial had high levels of FSH and LH which decreased with oestrogen therapy and significantly more so when an oestrogen-progestogen mixture was taken. However, a direct relation between the circulating oestrogen and gonadotrophin levels is more difficult to show. Before treatment, the correlation was significant between FSH and E_1 ($r=-0.44$ in the 49 patients; correlations greater than ±0.29 are significant at $p<0.05$ for pretreatment occasions). However, for the 41 patients receiving HRT, the correlation between FSH and E_1 was not significant ($r=-0.09$; correlations greater than $r=\pm0.32$ are significant at $p<0.05$ for the HRT group). The same was true between FSH and E_2 ($r=-0.49$ pretreatment; $r=-0.29$ post-treatment).

The most likely explanation is that in the untreated menopausal woman the range of oestrogen and gonadotrophin levels is relatively narrow and a degree of correlation is found. Therapy induces changes in the expected direction for both of these groups of hormones but the range of values both within patients from occasion to occasion and across patients is so variable that no convincing cause and effect relation can be demonstrated.

Oestrone and oestradiol

The wide standard deviations of the pretreatment oestrone and oestradiol levels were largely due to the high endogenous values found in a small number of patients. It is also appreciated that the blood samples reflect concentrations at a specific time point and cannot be assumed to reflect a 'steady-state' over the 24 hours. Accepting these limitations, oestrone showed an increase in concentration from pretreatment to treatment values in all 41 patients on HRT and a subsequent decrease in all patients after therapy was discontinued. Oestradiol increased in 38 of the 41 patients on HRT and decreased three months after therapy was stopped in 37. However, despite the type of oestrogen preparation taken, oestrone concentrations were *invariably* higher than the oestradiol levels within patients.

In the untreated state there was a close correlation between E_1 and E_2 ($r=0.89$ for 49 patients); this was reduced on HRT ($r=0.64$ for the 41 patients taking HRT).

With the small numbers of patients available and the wide range of values for E_1 and E_2 in the pretreatment state, no statistical conclusions can be drawn concerning the change in circulating levels when given alone or in combination with a progestogen. However, Tables XIII and XIV show an apparent reduction in the level of both oestrone and oestradiol when oestrogens are given in combination with a progestogen than when given alone. If such a change were shown to be true, then the benefits of the combined preparation described may not result from the effective plasma concentration of either oestrone or oestradiol but could be due to either a direct effect of the progestogen or to the ratio of the progestogen to oestrogen concentrations achieved in the circulation.

It seems that the rate of absorption of hormonal preparations taken orally is erratic both across patients and within patients from occasion to occasion. As demonstrated by the data in Tables XVII and XVIII, some patients appear to be absorbing the hormones within the first hour of ingestion while others show no change after two hours. Even when absorption does appear to take place, shown by a rise in the serum oestrogen levels, the serum progestogen concentration does not necessarily increase proportionally—or indeed at all in some instances. However, it does seem that, in general, the blood sample obtained in the fasted state and before any tablet has been taken (Table XVII) has a much higher level of oestrone and oestradiol than would occur in untreated women. *Thus one tablet per day elevates the basal level over the 24 hours* with further increases presumably for a few hours *each day* after a further pill is taken.

There was no correlation between the blood levels of oestrogen on the one hand and either the symptomatic improvement obtained, the vaginal cytological picture or the severity of menstruation induced on the other hand. Nor were there any significant side effects from any preparation. Some patients complained of a 'nasty' taste in the mouth after two or three months on HRT and others of a 'bloated' abdominal sensation. However, none of these symptoms discouraged the patients from continuing therapy. It must also be remembered that the majority of women in the placebo group obtained some relief of menopausal symptoms so the 'placebo' effect of actual therapy could also be present and make it difficult to decide if any one form of HRT was significantly better than another in terms of symptomatic relief obtained. Accepting the placebo effect, however, there could be no clinical doubt that HRT did alleviate the sensation of hot flushes and the real problem of severe perspiration completely, and thus greatly enhance the quality of life of those women suffering from these distressing symptoms.

Progestogens

From the few data available it appears that the absorption rate of norethisterone acetate and norgestrel is not uniform within patients from occasion to occasion; nor does it appear to relate to the rate of absorption of oestrogens. Symptomatically the patients felt as well as patients taking an oestrogen alone but no better. Induced menstruation, while tolerated, was not welcomed by any patient but if no alternatives were available many women would no doubt accept this bleeding.

It could also be argued that the addition of a progestogen reduces the levels of serum cholesterol, triglyceride, FSH and LH more than oestrogens alone. However, while achieving statistical significance, the changes are small and unlikely to be of therapeutic value. The fact that progestogens may also reduce the average circulating level of the oestrogens means that a small dose of an oestrogen alone could be prescribed to achieve equivalent plasma concentrations. This, in turn, would reduce any potential side effects from oestrogens, particularly the unwanted effect of induced uterine bleeding. On balance therefore, from the symptomatic and therapeutic viewpoint, there would seem to be little to be gained from the addition of a progestogen. The cost of a low dose oestrogen preparation would be cheaper than a combined oestrogen-progestogen preparation and the clinician would be left with the valuable warning sign of postmenopausal bleeding. That progestogens may reduce the risk of endometrial carcinoma is at best an unproven hypothesis; if it is believed that oestrogens may induce endometrial carcinoma then it would seem better not to prescribe them at all.

Haematological indices

The fall in haemoglobin and haematocrit which occurred in the majority of women resulted from a decrease in the number of red cells. As the MCV was not affected, it seems unlikely to be a problem of iron-deficiency but could result from a marginal slowing in the rate of red cell production. Recently it has been shown that the red cell mass decreases during the first trimester of pregnancy (Taylor and Lind, 1979); this might result from the fact that average circulating oestrogen levels are above those found in the luteal phase of the menstrual cycle. During the menopause, women presumably adjust to a low basal oestrogen level and the sustained increase achieved with HRT may be proportionally sufficient to induce a similar effect upon red cell production. However, in the clinical sense this change is too small to constitute a contraindication to the use of HRT.

Clotting factors

The data fail to reveal any increased tendency towards intravascular coagulation. Indeed if anything, a few women tended to show a haemorrhagic tendency in the sense of severe uterine bleeding. While low doses of synthetic compounds may produce equivalent symptomatic relief, it would need to be shown by similar studies that they were equally safe with respect to clotting, particularly in women of this age group.

The list of investigations undertaken in this study is by no means comprehensive; calcium balance studies, plasma enzyme concentrations, detailed liver function tests and many other factors would have been of interest. However, the volume of blood obtained and the number of procedures asked of each patient had to be carefully modulated to maintain maximum co-operation over the 10 months that each patient was studied.

Many of the symptoms which the patients discussed during their interviews have not been reported because they were difficult to quantify. Vaginal dryness and dyspareunia have been reported to be particularly related to 'hormone deficiency'; however, in many patients these two symptoms do not appear to be related. Thus, some women complained of dyspareunia while clinically the vagina seemed moist and elastic; in others the smooth vagina clinically associated with 'hormone deficiency' was not related to either the serum hormone levels present or any complaint of dyspareunia. Women who responded well to HRT in that they had more energy, fewer headaches or less depression often said that intercourse was more satisfactory; but this obviously reflected more changes than just their vaginal status. It was hoped that vaginal cytology, a more quantifiable characteristic, would be related to either symptoms of 'dryness', 'dyspareunia' or hormonal status. As this did not prove to be the case there seemed little point in attempting to correlate less measurable parameters with HRT.

POST-TRIAL FOLLOW-UP

After the three months off all therapy to complete the trial, each woman was offered the chance to continue with HRT if she wished but was told that she would be randomized into one of the other treatment groups. Of the 49 women completing the trial, only 22 availed themselves of this offer (Table XXIII), the commonest reasons for not taking advantage of further treatment were either than the return of bleeding did not balance the symptomatic relief or that the symptoms experienced before the trial were not now troublesome. Virtually every woman had had some return of symptoms after HRT was discontinued but usually not in so severe a form. While it is not possible to quantify such clinical impressions, it did seem that the 22 volunteers were those who experienced the same degree of discomfort from flush attacks and perspiration, loss of energy and irritability as before the trial. Interestingly, 3 of the 8 women given a placebo felt the improvement gained on these tablets was sufficient to wish to go on with therapy.

Each patient was told that after six months on the second form of therapy a formal uterine currettage would be performed under general anaesthesia as an in-patient. After obtaining their informed consent each was then randomly allocated to a different therapy group and reviewed each month thereafter. Their details are given in Table XXIV.

Each of the 3 women women originally given a placebo felt symptomatically much worse when given hormones. Indeed the symptoms were sufficient for them to discontinue all therapy after only two months.

With only one exception, the women given an oestrogen-only preparation orginally preferred this to the second therapy; this was usually because when randomized onto a combined preparation they did not feel that the symptomatic improvement was worth the return of menses. In the case of the patient who did prefer the second therapy (Patient 5, Table XXIV) this was because uterine bleeding induced by the original therapy did not occur with the second.

The converse was usually true of those women originally given a combined preparation; they usually preferred the second therapy if this did not induce menses or they found the first and second therapies to be equivalent when bleeding occurred on both. There were two exceptions; one patient felt symptomatically better on a combined preparation despite the fact that this induced uterine bleeding while her oestrogen-only second treatment did not (Patient 15, Table XXIV). The second patient found that a placebo given as second therapy did not produce as much symptomatic relief and neither treatment induced menstruation.

Sixteen patients completed a second six months on therapy and underwent uterine currettage under general anaesthesia. This was done on the seventh day off therapy following the sixth month on hormones. The choice of this time was deliberate as it seemed more logical to investigate the basal endometrial status in the seven days off therapy than to study the histological situation under the influence of circulating hormone levels which, from our data, could be erratic from time to time in the same patient. If therapy is going to have an adverse effect on the endometrium, it seems reasonable to suppose that such effect would be present in some measure between therapy courses.

At the time of the currettage, four patients had taken EO and one was menstruating regularly,

TABLE XXIII

Numbers of patients wishing to continue with HRT after completion of the trial according to the therapy taken

	Therapy taken during the trial						
	Placebo	EO	EO + NA	E_1S	E_1S + NA	E_2	E_2 + Ng
Number in original group (n = 49)	8	6	8	7	7	7	6
Number wishing to continue (n = 22)	3	2	4	5	3	3	2

TABLE XXIV

Clinical details of 22 patients electing to have a second course of HRT and whether or not dilation and curettage (D and C) performed

Patient	Original therapy (1)		Second therapy (2)			D and C performed	Comments
	Type	Menses induced	Type	Symptoms better on (1) or (2)	Menses induced		
1	Placebo	No	E_2	(1)	No	No	Felt awful on (2). Discontinued at 2 months
2	Placebo	No	E_2	(1)	Yes	No	Heavy 'periods' on (2). Discontinued at 2 months
3	Placebo	No	E_1S	(1)	No	No	Felt awful on (2). Discontinued at 2 months
4	EO	No	EO + NA	(1)	No	Yes	Returned to (1) after 3 months. Improved
5	EO	Yes	E_1S + NA	(2)	No	Yes	Patient preferred no menses on (2)
6	EO + NA	Yes	E_2 + Ng	(1) and (2) equal	Yes	Yes	Equally content on (1) or (2)
7	EO + NA	Yes	Placebo	(2)	No	No	Symptomatic relief on (2); no menses preferred
8	EO + NA	Yes	E_2	(2)	No	Yes	Preferred no symptoms
9	EO + NA	Yes	E_2	(1) and (2) equal	Yes	Yes	Equally content on (1) or (2)
10	E_1S	No	E_1S + NA	(1)	Yes	Yes	Preferred not to have menses
11	E_1S	No	E_2	(1) and (2) equal	No	Yes	Equally content on (1) or (2)
12	E_1S	No	E_2	(1)	No	Yes	Changed back to E_1S after 3 months
13	E_1S	No	EO + NA	(1)	Yes	Yes	Changed back to E_1S after 3 months
14	E_1S	No	E_2 + Ng	(1)	Yes	Yes	Changed back to E_1S after 3 months
15	E_1S + NA	Yes	E_2	(1)	No	Yes	Symptomatically not as well but no menses
16	E_1S + NA	No	Placebo	(1)	No	Yes	Changed back to E_1S + NA after 2 months
17	E_1S + NA	Yes	EO	(2)	No	Yes	Symptomatically as good; no menses
18	E_2	No	EO	(2)	No	Yes	Marginal improvement on (2)
19	E_2	No	E_1S + NA	(1)	Yes	Yes	Menses painful; back to E_2 after 2 months
20	E_2	No	E_1S	(1)	No	No	Abdominal pain; stopped all therapy after 2 months
21	E_2 + Ng	Yes	EO + NA	(1) and (2) equal	Yes	No	Discontinued all therapy after 3 months
22	E_2 + Ng	Yes	EO	(2)	Yes	Yes	Menses much less on (2)

three had taken E_1S and none had menses, five had taken E_2 and one bled regularly, three had taken E_1S+NA and one bled regularly while one had taken E_2+Ng and also bled regularly. As six of these patients had reverted to their primary therapy before curettage because of the induced uterine bleeding (Table XXIV), the group was self-selected.

Only two patients yielded any endometrium and then only tiny fragments. One patient, taking oestrone sulphate, showed a normal non-secretory endometrial pattern while the other, on oestradiol and norgestrel, showed a normal secretory phase picture.

At the end of the second period of six months only one patient wished to continue with HRT; thus out of the 49 patients completing the trial only this one patient felt that she still required the benefit of HRT 15 months from first beginning such therapy.

CONCLUSIONS

1. Less than half of all the women approached wished to avail themselves of the use of HRT despite a more personal service being offered than that available in an ordinary out-patient clinic such as personalized appointments and transport to and from their homes. While it could be argued that the population approached was not representative of the country as a whole, this is balanced by the fact that many of those who did volunteer would not have sought advice under normal circumstances. It would therefore seem that severe menopausal symptoms are uncommon or of short duration.

2. Some women have high endogenous oestrogen levels up to 18 months from the cessation of spontaneous menstruation. If it is believed that the purpose of HRT is to increase circulating oestrogen levels above the menopausal basal level, then it would seem pointless to give women oestrogens by mouth until their endogenous blood levels have been assayed. Vaginal cytology alone would be a poor indicator.

3. Following from this it could be argued that in the endogenous state, both premenstrually and postmenstrually, oestradiol is the dominant oestrogen; after HRT, oestrone predominates. As this appears to offer symptomatic relief the possibilities are (i) taking any pill, placebo or otherwise, is helpful; (ii) oestrone levels dictate the symptomatic state; or (iii) the oestradiol to oestrone ratio is in some way responsible for the symptomatic state.

4. While levels of FSH and LH are reduced on HRT, neither is decreased to anywhere near the premenopausal average level; FSH is decreased proportionally more than LH. Prolactin levels are not stimulated.

Lucky *et al* (1979) have reported that LH levels measured by immunoassay do not always reflect the potency of this hormone when determined by bioassay under varying physiological conditions; it is possible that exogenous oestrogens alter the relation of bioactive to immunoreactive LH. It would be interesting to know whether the relatively small lowering of FSH and LH levels caused by HRT, as determined by immunoassay, was masking a more profound change in the biopotency of these hormones and hence help to explain the symptomatic relief obtained.

5. The above points indicate that the term 'hormone replacement therapy' is a misnomer. The giving of HRT does not return a woman to anything like the endocrinological status of the premenopausal years nor is she physiologically similar to her untreated menopausal counterpart. Perhaps such therapy ought to be called 'hormone induction therapy' to indicate clearly that such women are in a new and induced condition which differs from the physiological premenopausal and postmenopausal states and which may have consequences which require much further investigation.

6. While a placebo has some effect, there is no doubt that women experiencing severe flushing attacks and perspiration do obtain significant relief with HRT. It is not clear why these attacks occur as their presence seems unrelated to the endogenous oestrogen status of the woman nor is her relief dependent on the induced oestrogen levels attained.

7. An added progestogen produces greater physiological changes than oestrogens alone, such as a lower cholesterol and triglyceride level in the circulation as well as lower concentrations of FSH and LH. However, it may also lower the level of circulating oestrogens in some women and this factor must be weighed against the effect the therapy is supposed to achieve. If these lower oestrogen levels are symptomatically as effective then they could equally well be achieved by a smaller dose of oral oestrogens presumably reducing the risks, if any, of these preparations.

8. Induced uterine bleeding is unacceptable to most women when they realize there is an alternative. It seems that women appreciate 6 to 12 months of symptomatic relief after which most will have experienced some permanent benefit. The addition of a progestogen produces further physical changes while offering no guarantee of regular endometrial shedding in *all* women. And in those who do bleed the loss may be so severe as to preclude continuing with therapy.

But are these dangers? Recent publications have suggested an increased incidence of endometrial carcinoma in women taking oestrogens (Ziel and Finkle, 1975; Antunes *et al*, 1979). Correspondence following these publications have dwelt on various postulated deficiencies in the project designs, the form of data analysis and the selection of patients. While these criticisms may have some validity they do not, of themselves, refute the possibility that prolonged oestrogen usage may increase the incidence of endometrial cancer. The protective effect of progestogens is also questioned in the recent paper by Jick *et al* (1979) in which it was reported that 7 out of 42 cases of such cancer occurred in women taking a combined preparation.

No data published to date have suggested any risk to women taking HRT for less than 12 months. It would seem reasonable, therefore, for doctors to allow women symptomatic relief without the disadvantage of uterine bleeding by prescribing a short course of oestrogens, namely, six months initially with a further period of six months if the patient requests it. There would seem no reasonable alternative to performing a diagnostic curettage thereafter and perhaps repeated every 12 to 18 months if HRT is maintained, to determine the histological status of the endometrium.

9. The use of a progestogen-only pill would seem to be worth investigating. As these preparations appear to potentiate the effect of oestrogens it may be that endogenous oestrogens can yield symptomatic relief if aided by an exogenous progestogen.

10. As the effect of HRT appears to be to raise the circulating levels of oestrone more than oestradiol, independently of the compound taken by mouth, there would seem to be nothing to choose between those preparations containing conjugated equine oestrogens, piperazine oestrone sulphate and oestradiol valerate. Thus the lowest dose of the cheapest product would seem to be first choice for the prescriber.

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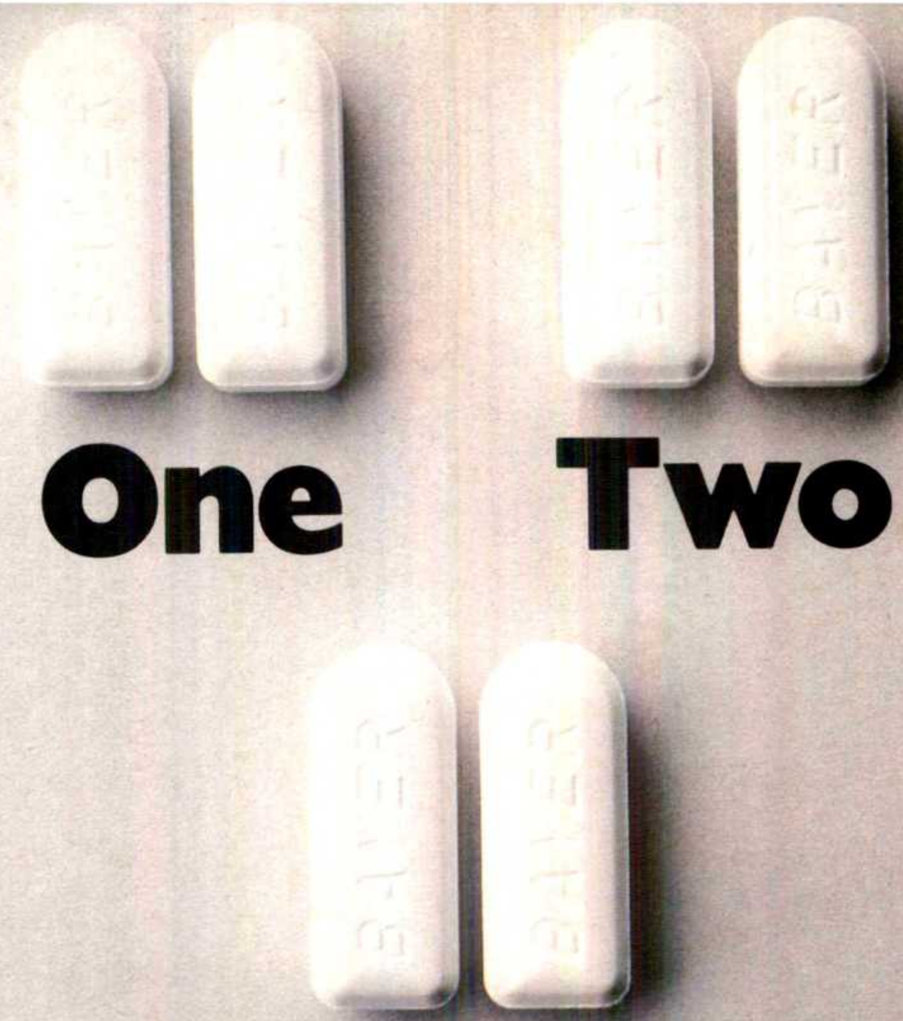

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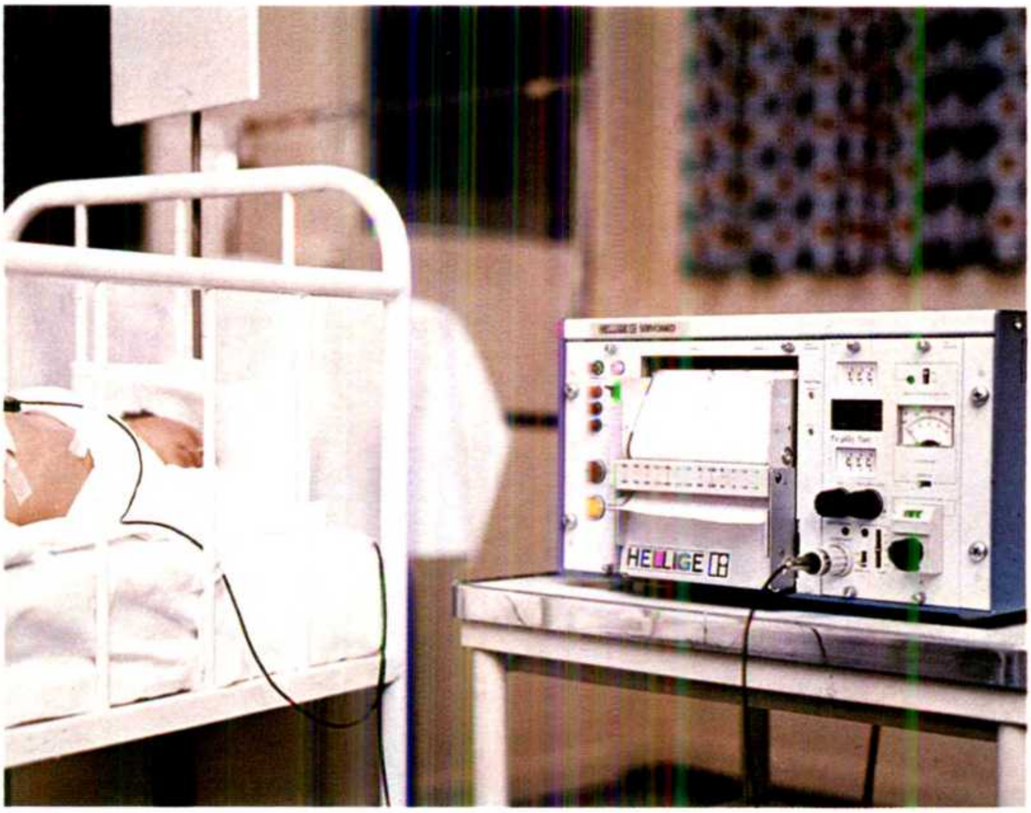

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Proper scientific names giving genus and species should be italicized, with an initial capital and contraction for the genus only after a full spelling at the first mention, thus: *Clostridium welchii* followed by *Cl. welchii*; *Escherichia coli* by *E. coli*.

Each sentence should have its proper components, thus: '*Progress:* The patient continued to improve', NOT '*Progress:* Went downhill'.

Numbers up to ten should be spelt, unless they are contrasted with other numbers. Larger numbers should be in the form of numerals and not words except when beginning a sentence, thus 'Fifteen patients out of a total of 60 exhibited'.

The name(s) of author(s) and their appointments at the time of preparation of the text should appear at the head of the paper. In the case of multiple authorship, the manuscript *must be* accompanied by a covering letter bearing the signatures of all the authors.

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These should be avoided as far as possible. 'Per cent' should be used instead of the symbol '%' and such forms as TB, RBC, BP, PET, APH eschewed. Dates should be given as 10th May, 1974 and *not* 10.V.74. The official rather than the proprietary names of drugs must be used.

The Journal convention is C for Celsius, m for metre(s), g for gram(s), A for ampere(s), K for kelvin(s), cd for candela(s), mol for mole(s), l for litre(s) and '/' for the divider 'per'. Submultiples of units are formed by prefixes and authors should use d for deci or 10^{-1}, c for centi or 10^{-2}, m for milli or 10^{-3}, μ for micro or 10^{-6}, n for nano or 10^{-9}, p for pico or 10^{-12}, f for femto or 10^{-15} and a for atto or 10^{-18}; for multiples the prefixes should be T for tera or 10^{12}, G for giga or 10^{9}, M for mega or 10^{6}, k for kilo or 10^{3}, h for hecto or 10^{2}, and da for deca or 10^{1}. Thus it would be kg for kilogram(s), ml for millilitre(s), μg for microgram(s), etc. Symbols should be typed without a full stop. In the writing of large numbers, groups of three figures should be separated by a space and not a comma (to avoid confusion with decimal points).

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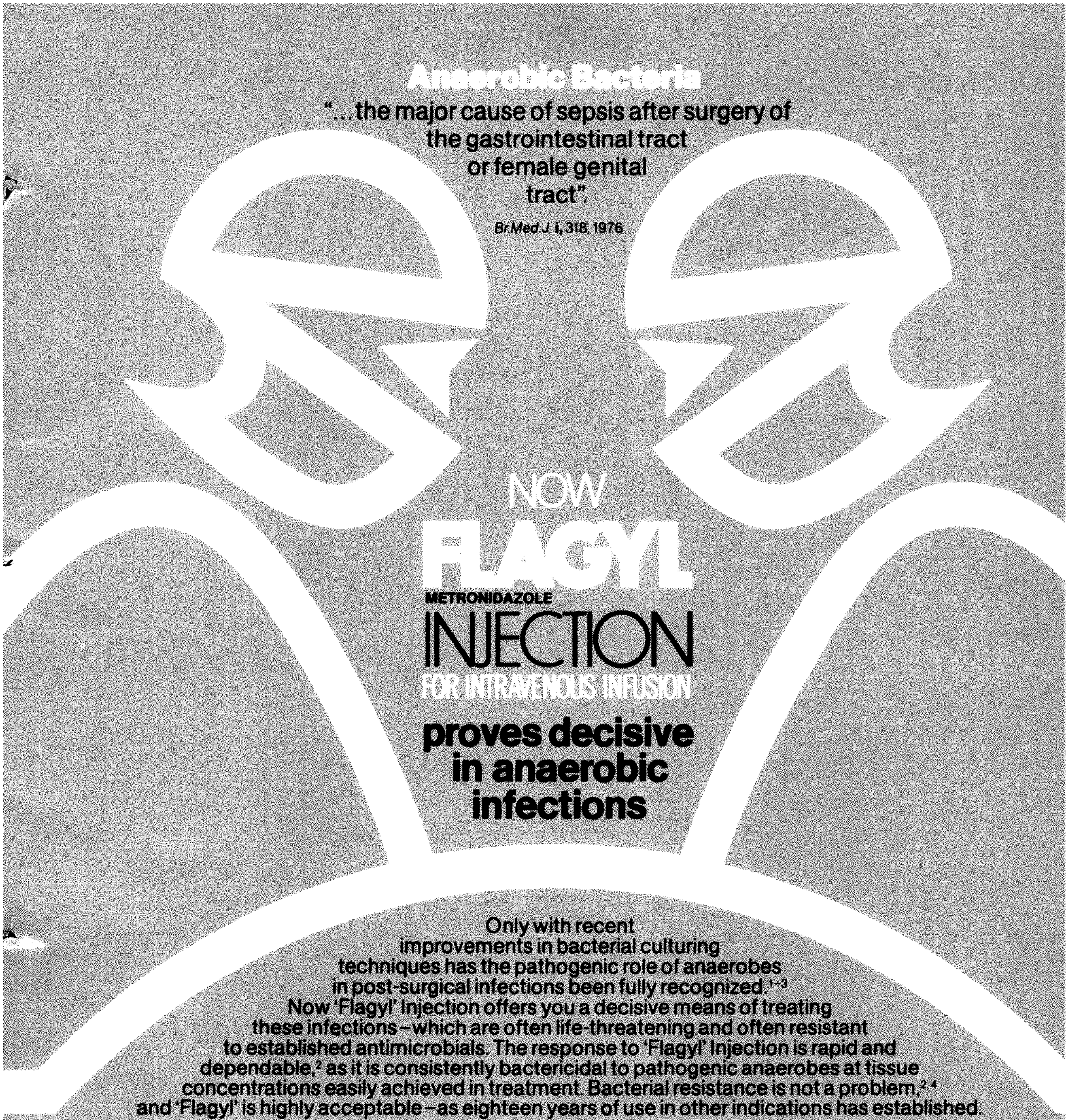

Anaerobic Bacteria

"...the major cause of sepsis after surgery of the gastrointestinal tract or female genital tract".

Br.Med.J. **i**, 318, 1976

NOW

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INJECTION

FOR INTRAVENOUS INFUSION

proves decisive in anaerobic infections

Only with recent improvements in bacterial culturing techniques has the pathogenic role of anaerobes in post-surgical infections been fully recognized.[1-3] Now 'Flagyl' Injection offers you a decisive means of treating these infections–which are often life-threatening and often resistant to established antimicrobials. The response to 'Flagyl' Injection is rapid and dependable,[2] as it is consistently bactericidal to pathogenic anaerobes at tissue concentrations easily achieved in treatment. Bacterial resistance is not a problem,[2,4] and 'Flagyl' is highly acceptable–as eighteen years of use in other indications has established.

Dosage: Treatment: adults and children over 12 years: 100 ml by intravenous infusion eight-hourly, administered 5 ml per minute. Oral medication with 400 mg three times daily should be substituted as soon as this becomes feasible. Treatment for seven days should be satisfactory in most cases. Children under 12 years: as for adults but the single intravenous dose is based on 1.5 ml (7.5 mg metronidazole) per kg bodyweight and the oral dose on 7.5 mg per kg bodyweight. Prevention: adults and children over 12 years: 100 ml by intravenous infusion immediately before, during or after operation, followed by the same dose eight-hourly until oral medication (200 to 400 mg three times daily) can be given to complete a seven-day course. Children under 12 years: as for adults but the single intravenous dose is based on 1.5 ml (7.5 mg metronidazole) per kg bodyweight and the oral dose on 3.7 to 7.5 mg per kg bodyweight. Precautions: pregnancy; lactation; clinical and biological surveillance if recommended duration of treatment exceeded; dosage may be halved for patients with renal failure; avoid alcohol; if 'Flagyl' is to be given to patients receiving oral anticoagulants the dosages of the latter should be recalibrated. Side effects and adverse reactions: occasionally an unpleasant taste, furred tongue, nausea, vomiting (very rarely), gastro-intestinal disturbance. Drowsiness, dizziness, headache, ataxia, skin rashes, pruritus, inco-ordination of movement, darkening of the urine very rarely. During intensive and/or prolonged therapy, peripheral neuropathy has been reported. A moderate leucopenia has been reported but the white cell count has always returned to

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Tablets 200 mg	PL 0012/5256
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Injection 0.5% w/v	PL 0012/0107

Basic N.H.S. cost (as at November 1978)
Injection for i.v. infusion Bottle of 100 ml £6.40.

References 1. Willis, A.T. (1977) Scottish Medical Journal, **22**, 155. 2. Willis, A.T. et al. (1977) British Medical Journal, **i**, 607. 3. Finegold, S.M. Anaerobic Bacteria in Human Disease, Academic Press Inc. New York, 1977. 4. Willis, A.T. et al (1975) Journal of Antimicrobial Chemotherapy, **1**, 393, 1975.

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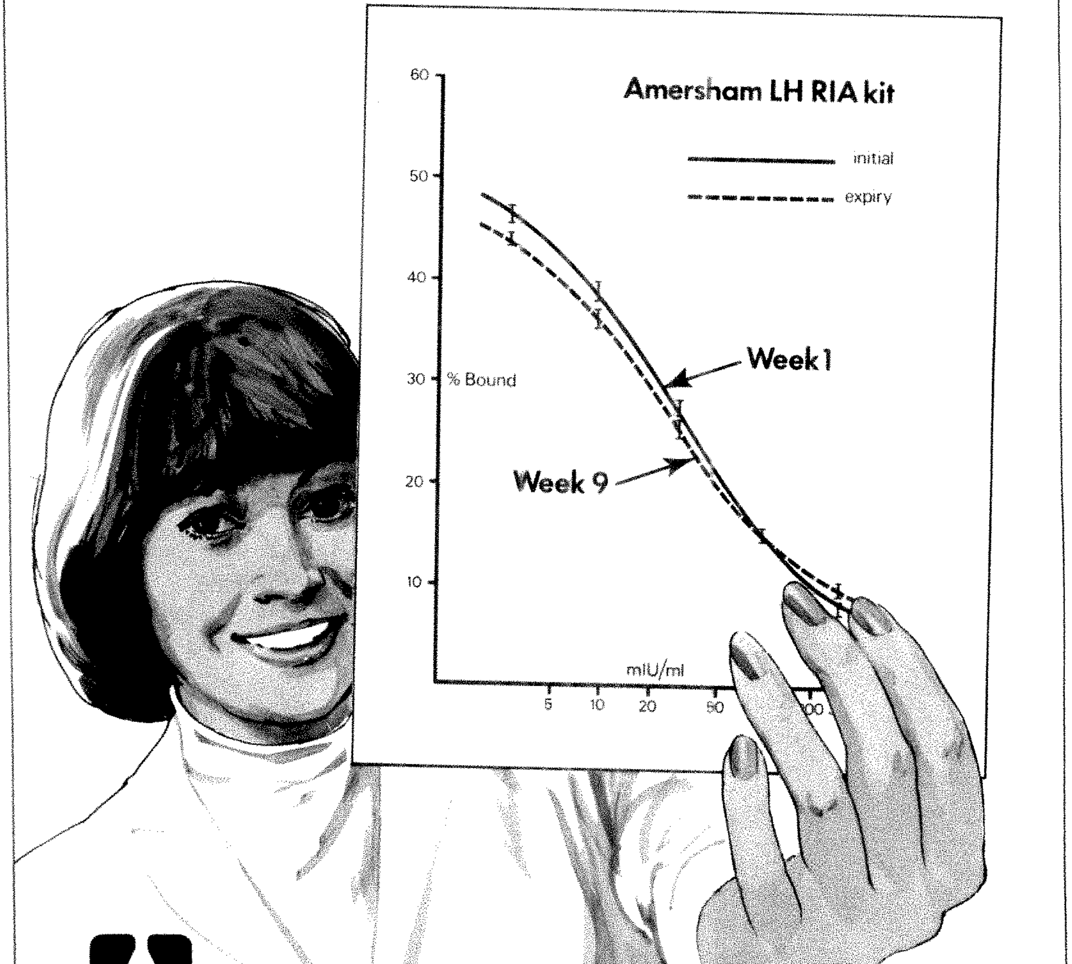

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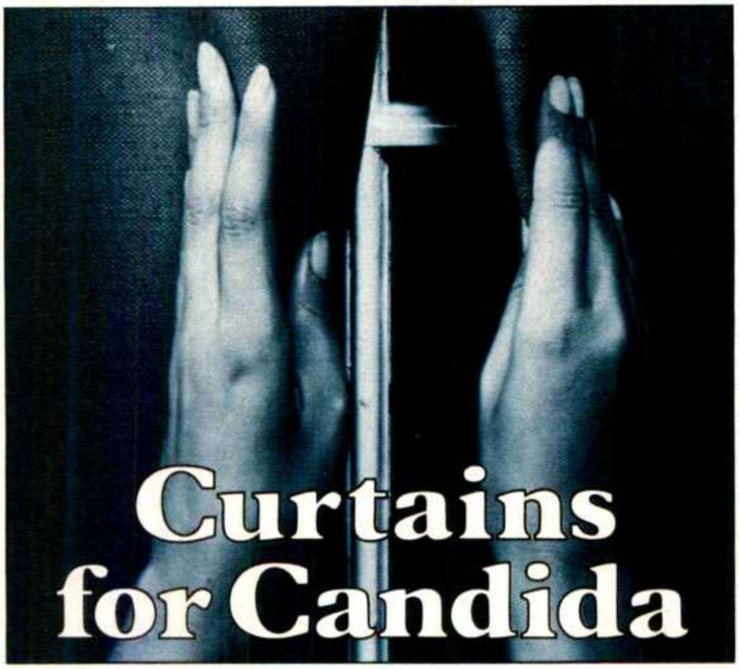

In just 3 nights

Ecostatin™

Econazole nitrate

Ecostatin™ eliminates candidal vaginitis in just 3 nights

- it is the most active fungicide against a wide range of pathogens[1]
- it combines potent fungistatic and fungicidal properties[2]

Ecostatin also ensures

- fast relief of symptoms
- no resistance[3]
- patient compliance

Ecostatin is also available as a cream for a wide range of dermatomycoses.

Prescribing Information
PRESENTATION
White, opaque oval pessaries each containing 150mg econazole nitrate in an hydrogenated vegetable oil base.
USES
Actions Econazole nitrate has a broad spectrum of antifungal activity. It is highly active against Candida albicans and other Candida species and is effective in controlling infections of the vagina and vulva caused by such organisms (thrush).
Indications Vulvovaginal candidosis.
DOSAGE AND ADMINISTRATION
One pessary to be inserted at bedtime for 3 consecutive nights. Administration should be continued even if menstruation occurs, and despite the disappearance of signs and symptoms of the infection. The pessary should be inserted high into the vagina while the patient is supine. Although a three day course of therapy usually suffices it may be necessary to institute a second course of therapy.
CONTRA-INDICATIONS, WARNINGS, etc.
Contra-indications None known.
Precautions Ecostatin pessaries are effective in the candidal vaginitis associated with pregnancy. However, as with other agents, it should not be used during the first trimester of pregnancy unless the physician deems it's use essential for the welfare of the patient.
Side Effects Very occasionally patients may complain of discomfort; this is usually transitory and disappears with continued treatment. Rarely is it necessary to discontinue econazole pessary treatment.
PHARMACEUTICAL PRECAUTIONS
Storage Store at room temperature.
LEGAL CATEGORY POM
PACKAGE QUANTITIES Packs of three pessaries with applicator.
Price – 3 pessaries with applicator £3.60
Product Licence Number 0085/0013.
References
1. Avram, A. et Grupper, Ch. Bulletin de la Société Française de Mycologie Médicale 1975 Tome IV (2).
2. Preusser, H.J. et al. Research Report 1974. On file Cilag-Chemie.
3. Schweisfurth, R. Research Report 1974. On file Cilag-Chemie.

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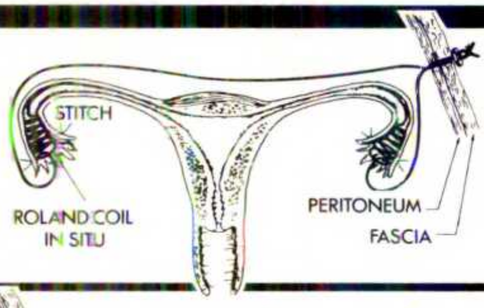
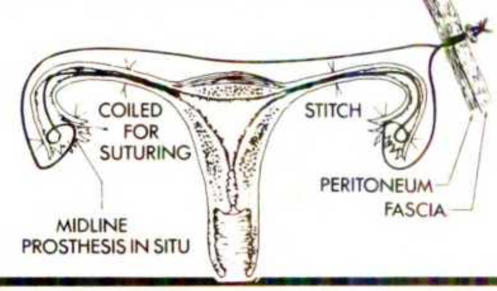
kli tuboplasty surgical prosthesis

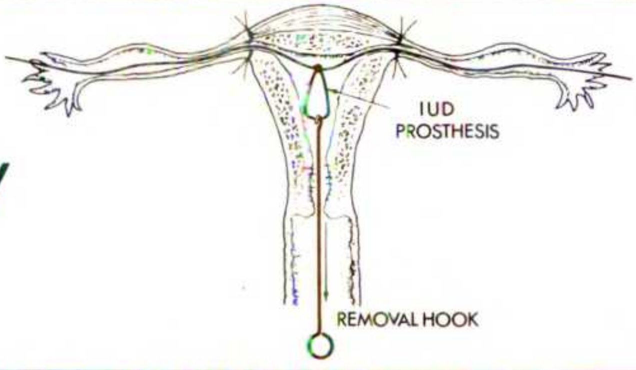
Stent for Fimbrial Tuboplasty





Stent for Midline Tuboplasty

Stent for Cornual Tuboplasty



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Prescribing Information

Presentation
White egg-shaped pessaries each containing 150mg econazole nitrate and melting at 37°C.

Uses
Vaginitis due to Candida albicans and other yeasts.

Dosage and Administration
One pessary should be inserted high into the vagina each evening for three consecutive days.

Contra-indications, Warnings etc.
Contra-indications: None known.
Side-effects: None known.
Overdosage: Not applicable.
Precautions: Hypersensitivity has never been recorded, but if it should occur, administration of the product should be discontinued.
Gyno-Pevaryl* 150 vaginal pessaries are effective in the treatment of vaginal candidiasis associated with pregnancy but as with any new product, caution should be exercised when prescribing during the first trimester.

Pharmaceutical Precautions
Store in a cool place.

Legal Category
Prescription only (POM).

Package Quantities
Each pack contains 3 pessaries and a patient instruction leaflet. The pessaries are individually sealed in a white plastic strip marked **Gyno-Pevaryl* 150** at regular intervals.

Further Information
Anogenital hygiene is important to help prevent re-infection.
PL0076/0058
Basic NHS Price – £3.60

Name and address of Licensee
Ortho Pharmaceutical Limited, P.O. Box 79,
Saunderton, High Wycombe,
Buckinghamshire
HP14 4HJ, England.

Further information and data sheet available on request.

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The quick way to say Gyno-Pevaryl*

Vaginal Pessaries

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The effective 3 day, 3 pessary treatment for vaginal candidiasis

Rapid symptomatic relief Burning pruritus and discharge promptly subside on therapy.
Effective in over 85% of cases – in only 3 days[1] Three days are all it takes to clear the condition in over 85% of cases.[1]
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Gyno-Pevaryl* 150

Vaginal Pessaries **Econazole Nitrate 150mg.**

new-generation antifungal

the quick way to treat vaginal candidiasis

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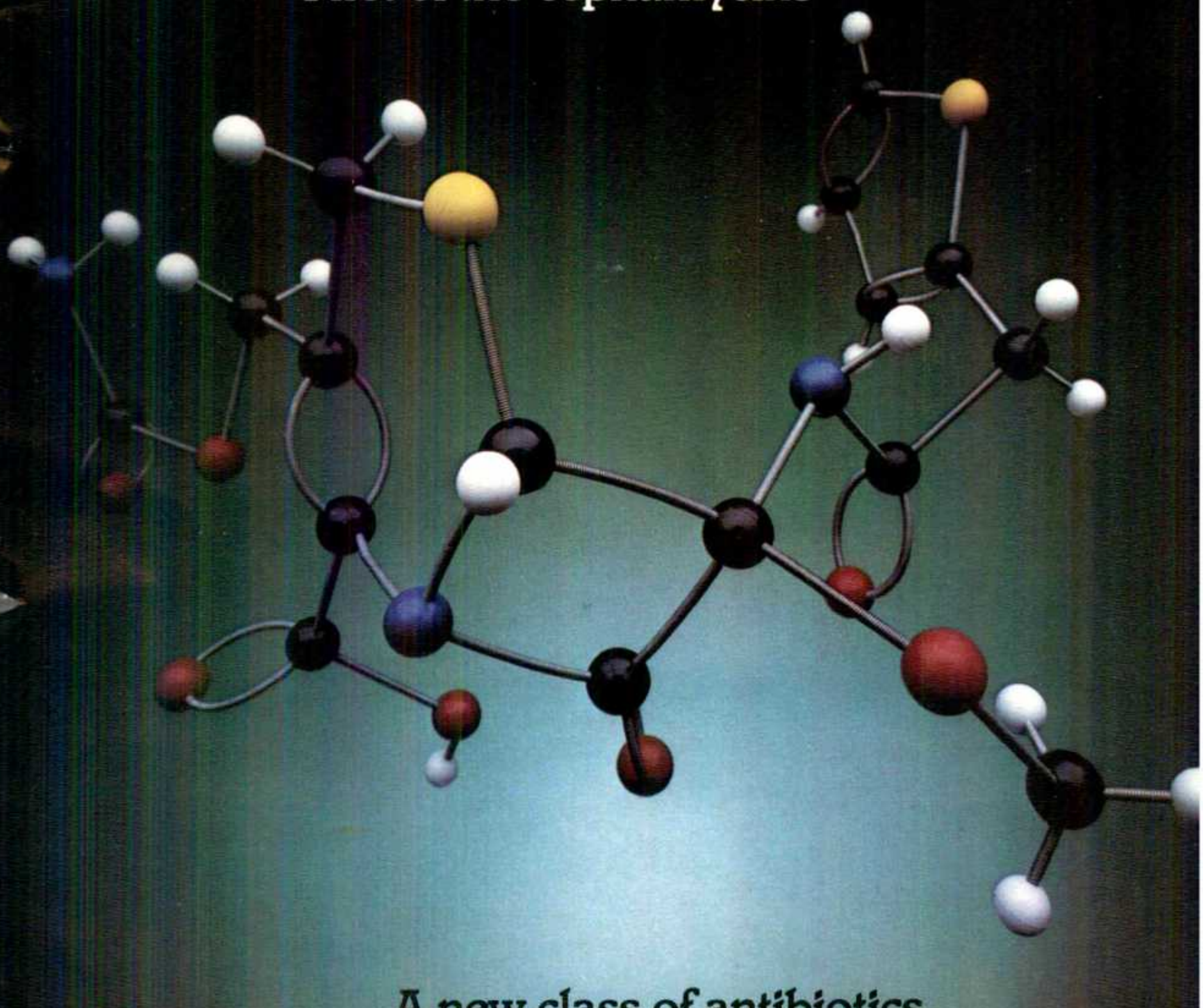
MSD announces
the discovery of

Mefoxin®

Cefoxitin sodium, MSD

INJECTION

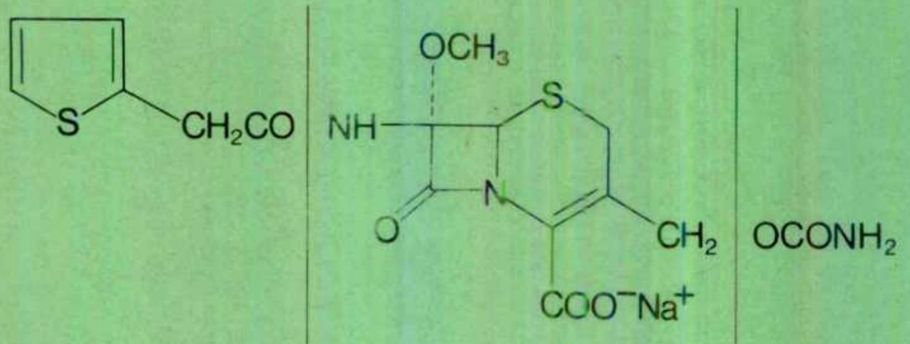
First of the cephamycins



A new class of antibiotics

'Mefoxin' is derived from cephamycin C, a naturally occurring antibiotic substance produced by *Streptomyces lactamdurans*. This naturally occurring antibiotic has been modified in the MSD Research Laboratories to enhance its therapeutic usefulness.

'Mefoxin' has a unique molecular structure offering outstanding clinical benefits and a specific clinical profile.



Thienylacetyl side-chain:

enhances activity against Gram-positive pathogens

Cephamycin nucleus:

provides bactericidal activity against a wide range of pathogens, Gram-negative in particular. 7α-methoxy group protects against β-lactamase degradation

Carbamoyloxy side-chain:

protects antibiotic from being metabolised

▼Prescribing information

Indications

'Mefoxin' is a broad-spectrum bactericidal antibiotic indicated for the treatment of infections caused by susceptible strains of Gram-positive and Gram-negative pathogens both aerobic and anaerobic.

'Mefoxin' has been clinically effective not only in infections due to antibiotic-sensitive organisms, but also in infections due to organisms resistant to one or more of the following antibacterial agents: penicillin, ampicillin, carbenicillin, tetracyclines, erythromycin, chloramphenicol, cephalosporins, kanamycin, gentamicin, tobramycin, and sulphamethoxazole-trimethoprim.

Many Gram-negative pathogens are resistant to penicillins and cephalosporins through the action of the β-lactamases which are produced by these pathogens. 'Mefoxin' is remarkably stable in the presence of these bacterial β-lactamases, both penicillinases and cephalosporinases. Hence, the clinical efficacy of 'Mefoxin' extends to many infections caused by such pathogens.

'Mefoxin' is indicated for the treatment of the following infections caused by sensitive bacteria:
Peritonitis and other intra-abdominal and intrapelvic infections; female genital tract infections; septicaemia; urinary tract infections; respiratory tract infections; bone and joint infections; and skin and soft-tissue infections.

'Mefoxin' is indicated for adjunctive therapy in the surgical treatment of infections, including abscesses, infection complicating hollow visceral perforations, cutaneous infections, and infections of serous surfaces, whether caused by aerobes, mixed aerobes and anaerobes, or anaerobes.

Microbiology

'Mefoxin' is active *in vitro* against:

Aerobic bacteria

Gram-positive cocci including: Staphylococci (including coagulase-positive, coagulase-negative, and penicillinase-producing strains); Group A beta-haemolytic streptococci *(Streptococcus pyogenes)*; Group B beta-haemolytic streptococci *(Streptococcus agalactiae)*; *Streptococcus pneumoniae (Diplococcus pneumoniae)*,

faecalis); *Gram-negative cocci including: Neisseria gonorrhoeae* (including penicillinase-producing strains); *Neisseria meningitidis*. *Gram-negative rods (facultative anaerobes) including: Escherichia coli; Klebsiella pneumoniae;* Klebsiella spp.; *Proteus mirabilis; Proteus* (indole-positive); *Proteus vulgaris; Proteus rettgeri; Proteus morganii; Haemophilus influenzae; Serratia marcescens;* Providencia spp.; Salmonella and Shigella spp.

Anaerobic bacteria

Gram-positive cocci including: Peptococcus spp., Peptostreptococcus spp., *Micro-aerophilic streptococcus*. *Gram-positive rods including: Clostridium perfringens*, Clostridium spp., Eubacterium spp., *Propionibacterium acnes*. *Gram-negative cocci including:* Veillonella spp. *Gram-negative rods including: Bacteroides fragilis, Bacteroides melaninogenicus*, Bacteroides spp. (including both penicillin-susceptible and penicillin-resistant strains), Fusobacterium spp.

'Mefoxin' is active against some strains of the following bacteria: Acinetobacter calcoaceticus var. anitratum *(Herellea vaginicola)*; Acinetobacter calcoaceticus var. lwoffi *(Mima polymorpha)*; Alcaligenes faecalis, Citrobacter spp. and Flavobacterium spp., Enterobacter spp.

'Mefoxin' is not active against Pseudomonas, most strains of enterococci and many strains of Enterobacter cloacae.

Dosage and administration

'Mefoxin' may be administered intravenously or intramuscularly. (See reconstitution directions for each route below.) Dosage and route of administration should be determined by severity of infection, susceptibility of the causative organisms, and condition of the patient.

Therapy may be started while awaiting the results of susceptibility testing.

Dosage: The usual adult dosage is 1 g or 2 g of 'Mefoxin' every eight hours. (See chart.)

In adults with renal insufficiency an initial loading dose of 1 g or 2 g

Usual adult dosage

Type of infection	Dose	Frequency	Total daily dosage
Uncomplicated	1 g	Every 8 hours (occasionally every 6 hours)	3 g (4 g)
Moderately severe or severe	2 g	Every 8 hours (occasionally every 6 hours)	6 g (8 g)
Infections generally needing antibiotics in higher dosage	3 g (2 g)	Every 6 hours (Every 4 hours)	12 g

Maintenance dosage of 'Mefoxin' in adults with reduced renal function

Renal function	Creatinine clearance (ml/min)	Dose	Frequency
Mild impairment	50-30	1-2 g	Every 8-12 hours
Moderate impairment	29-10	1-2 g	Every 12-24 hours
Severe impairment	9-5	0.5-1 g	Every 12-24 hours
Essentially no function	<5	0.5-1 g	Every 24-48 hours

In the patients undergoing haemodialysis, the loading dose of 1 to 2 g should be given after each haemodialysis, and the maintenance dose should be given as indicated in the table above.

Intravenous administration: reconstitute 'Mefoxin' with Water for Injection BP. 1 g is soluble in 2 ml. Although 'Mefoxin' is very soluble, for intravenous use it is preferable to add 10 ml of Water for Injection BP to the 1 g vial or the 2 g vial. Shake to dissolve and then

Outstanding clinical benefits

Wide-ranging activity against Gram-negative and Gram-positive pathogens, anaerobes as well as aerobes.

Unusually high resistance to β-lactamase degradation.

Highly active against *Bacteroides fragilis* and strains of *E coli*, Serratia, Klebsiella spp. and Proteus spp. (including indole positive strains) resistant to other antibacterial agents.

Offers high levels of active antibiotic in the body.

A record of relative safety comparable to the penicillins.

Prime indications include:

- Peritonitis, and other intra-abdominal and intrapelvic infections.
- Female genital tract infections
- Septicaemia
- Skin and soft tissue infections
- Bone and joint infections
- Urinary tract infections
- Respiratory tract infections (e.g. inhalation pneumonia)

Specific clinical profile

'Mefoxin' is exceptionally valuable wherever Gram-negative pathogens and anaerobes are involved in mixed infections. When mixed infections are suspected, therapy may be started while awaiting the results of susceptibility testing.

Mefoxin®

Cefoxitin sodium, MSD **INJECTION**

First of the cephamycins

the vein over a period of 3 to 5 minutes or may be given through the tubing when the patient is receiving parenteral solutions.

An intermittent intravenous infusion of 'Mefoxin' may be employed when large amounts of fluid are to be given. However, during infusion of the solution containing 'Mefoxin', it may be advisable temporarily to discontinue administration of any other infusion solution at the same site (by using an appropriate IV infusion set).

A solution of 'Mefoxin' may also be given by continuous intravenous infusion (see below for compatibility and stability).

Intramuscular administration ONLY
Reconstitute 'Mefoxin' 1 g with 2 ml of Water for Injection BP or 0.5% or 1% lignocaine hydrochloride (without adrenaline) solution. 'Mefoxin' is given by deep injection into a large muscle mass. Avoid injection into a blood vessel.

Compatibility and stability
A solution of 'Mefoxin' in Water for Injection BP may be added to the following solutions: 0.9% Sodium Chloride Injection BP, 5% or 10% Dextrose Injection BP, Dextrose and Sodium Chloride Injection (5%/0.9%, 5%/0.45%, or 4.3%/0.18%), Lactated Ringer's Injection USP, 5% Dextrose in Lactated Ringer's Injection, 5% or 10% invert sugar in water, 10% invert sugar in saline solution, 4.2% Sodium Bicarbonate Injection BP, M/6 Sodium Lactate Injection BP.

'Mefoxin', as constituted to 1 g/10 ml with Water for Injection BP, Water for Injection BP preserved with parabens or benzyl alcohol, 0.9% Sodium Chloride Injection BP or 5% Dextrose Injection BP maintains satisfactory potency for 24 hours at room temperature, for 96 hours under refrigeration, and for 13 weeks in the frozen state.

'Mefoxin' can be administered concomitantly with such antibiotics as gentamicin, tobramycin, carbenicillin or amikacin. When 'Mefoxin' is administered with another antibiotic, they should not be mixed in the same syringe.

Contra-indications
'Mefoxin' is contra-indicated in persons who have shown hypersensitivity to cefoxitin. In the absence of clinical experience,

Precautions
There is some clinical and laboratory evidence of partial cross-allergenicity between cephamycins and other β-lactam antibiotics, penicillins, and cephalosporins. Severe reactions (including anaphylaxis) have been reported with most β-lactam antibiotics.

Before therapy with 'Mefoxin', careful inquiry should be made concerning previous hypersensitivity reactions to β-lactam antibiotics. 'Mefoxin' should be given cautiously to penicillin-allergic patients.

Any patient who has demonstrated some form of allergy, particularly to drugs, should receive antibiotics cautiously. If an allergic reaction to 'Mefoxin' occurs, the drug should be discontinued.

The total daily dosage should be reduced when 'Mefoxin' is administered to patients with transient or persistent reduction of urinary output due to renal insufficiency (see 'Dosage and administration') because high and prolonged serum antibiotic concentrations can occur from usual doses.

Interference with laboratory tests
A false-positive reaction to glucose in the urine may occur with reducing substances but not with the use of specific glucose oxidase methods.

Use in pregnancy
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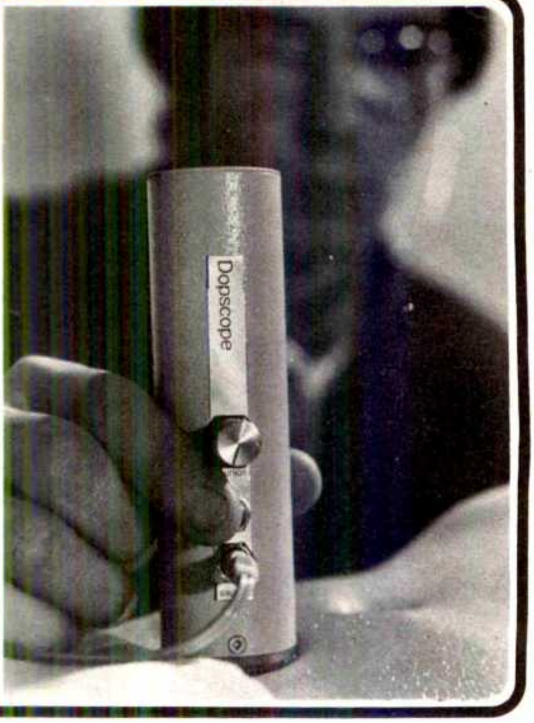

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1. J. int. med. Res. (1977) 5 (Suppl. 3): 86-91.
2. ibid., 101-108. 3. ibid., 98-100.
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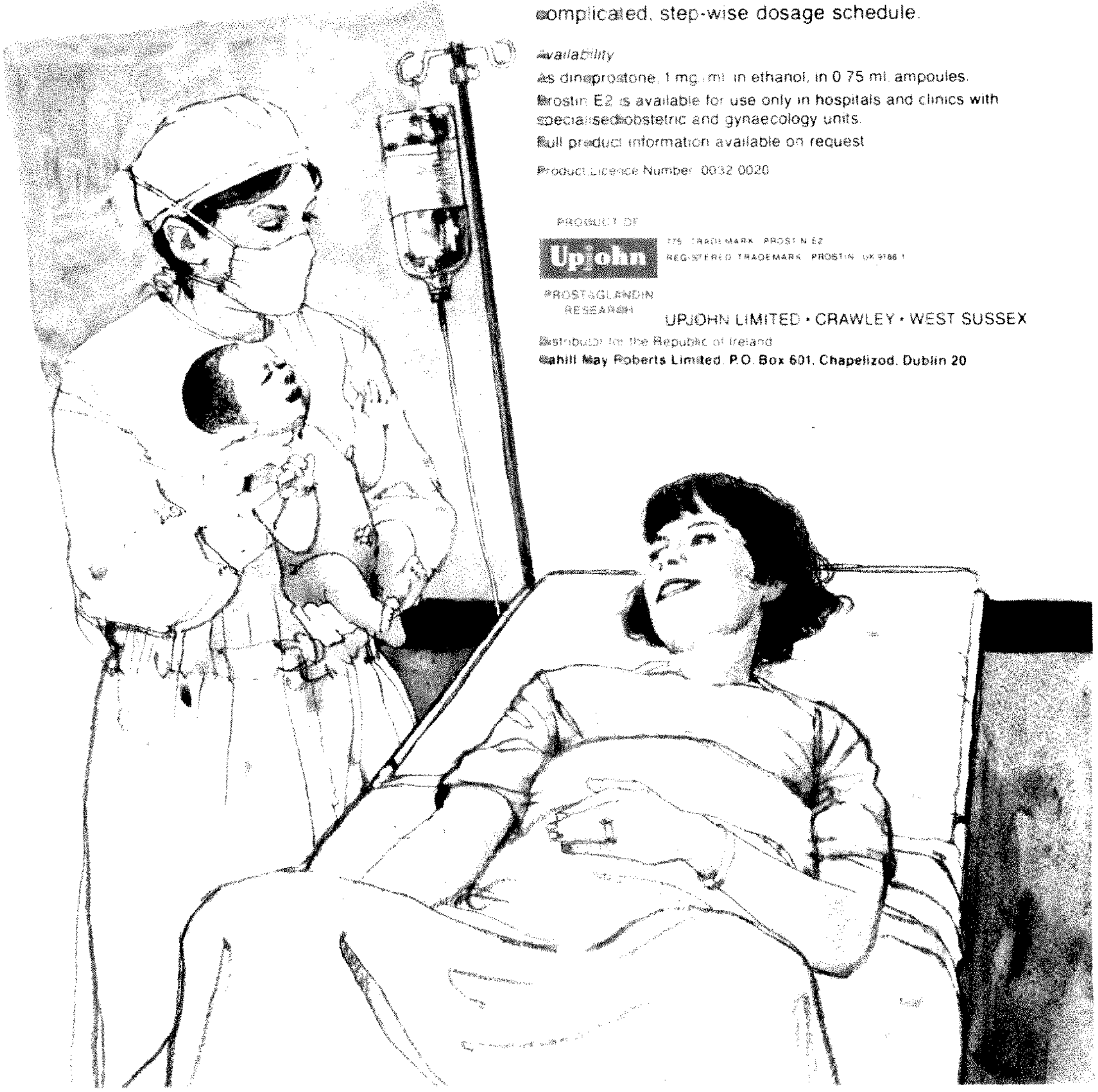

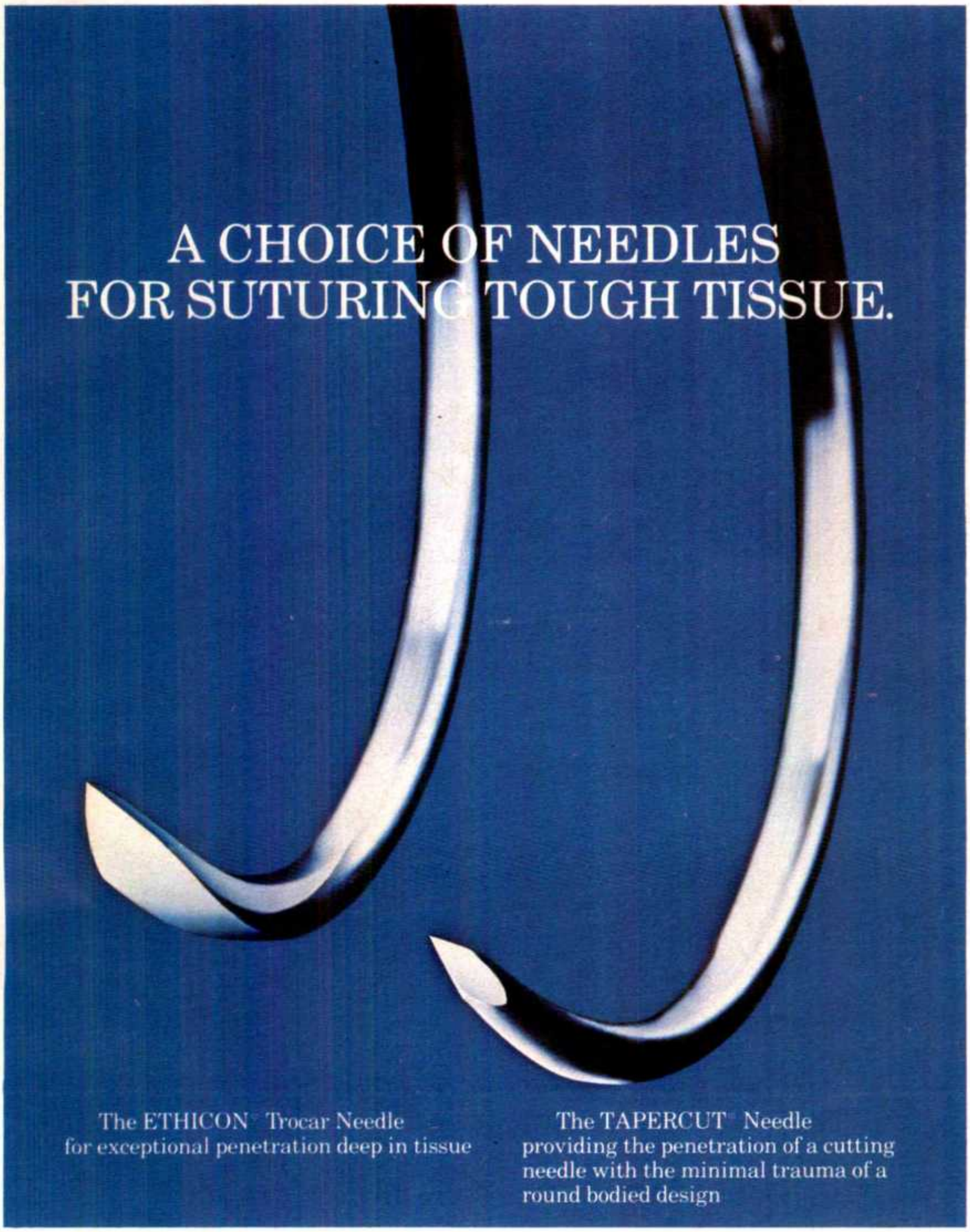

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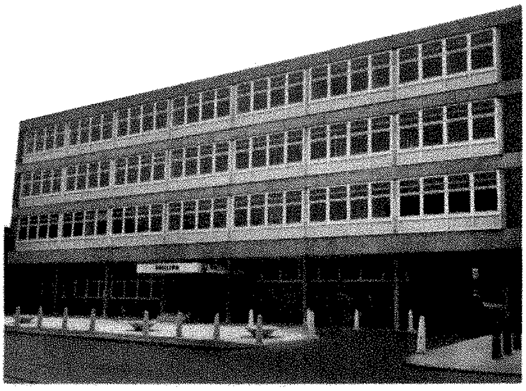

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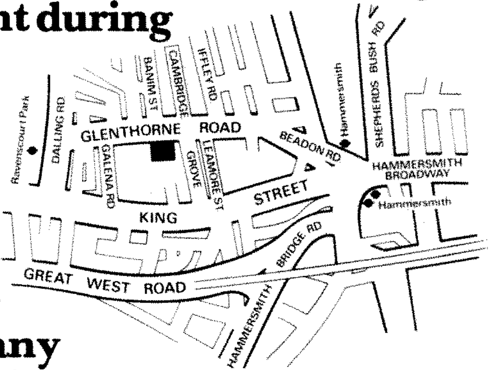

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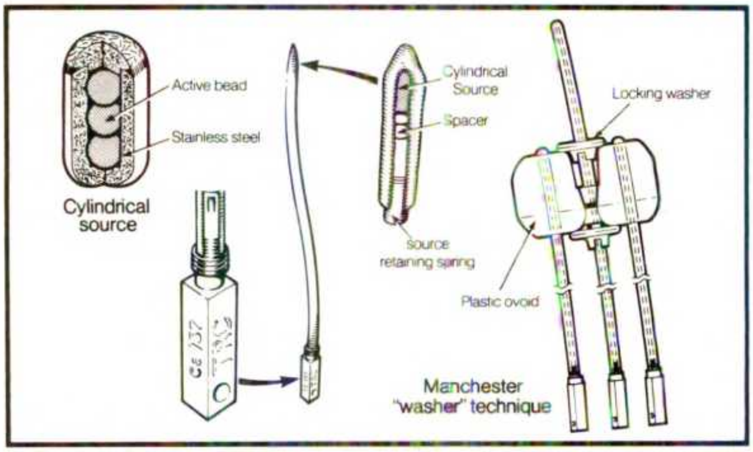

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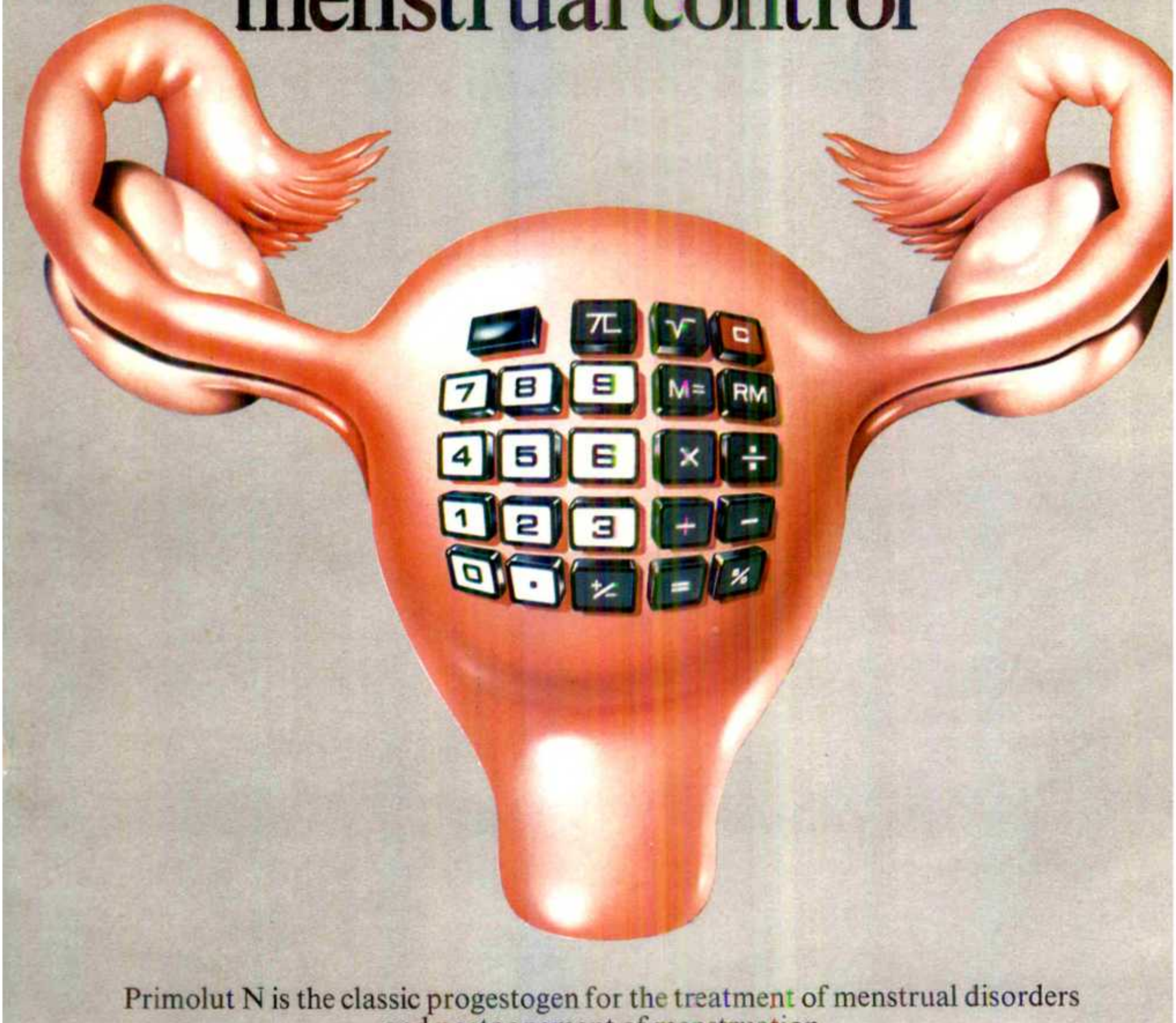

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British Journal of Obstetrics and Gynaecology

Vol 86 No. 4 NEW SERIES APRIL 1979

A RETROSPECTIVE STUDY OF INDUCTION OF LABOUR

BY

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AND
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University of Oxford, John Radcliffe Hospital, Headington, Oxford

Summary

The labour, delivery and state of the newborn were compared retrospectively in a group of women who had labour induced and a group who started labour spontaneously. There were 200 women at term in each group, individually matched for age, parity and social class and with no medical or obstetric complications prior to labour. The induced group showed a higher incidence of epidural anaesthesia, fetal monitoring and operative delivery. Sixty had forceps deliveries and five had Caesarean sections compared with two forceps deliveries and no Caesarean sections in the 'spontaneous onset' group. The higher incidence of operative deliveries in the induced group was not fully explained by the excess of epidurals and fetal monitoring received. More of the babies born after an induced labour had endotracheal intubation during resuscitation.

DISCUSSION about induction of labour centres on two aspects. The first is the possibility that induction can reduce perinatal mortality by preventing prolonged pregnancy. The second is the possible effect of induction on the individual woman in labour and on the state of the newborn infant.

We have studied this second aspect by investigating the results of obstetric practice in the John Radcliffe Hospital. The study is a retrospective one, which compares a group of women who had labour induced with a closely matched group who went into labour spontaneously. To further clarify comparison between the groups, women with any medical or obstetric abnormality have been excluded from the study.

METHODS

The study was concerned with patients who were delivered in the Consultant Unit of the John Radcliffe Hospital in 1975. In that year, the induction rate in the Consultant Unit was 35 per cent.

From the hospital lists of induced patients, a sample of 210 consecutive patients was chosen in whom induction had taken place, in the absence of any antenatal complications. Each induced patient was then matched for age (± 4 years), parity (number of previous livebirths at term) and social class (based on the husband's occupation) with a patient whose onset of labour had been spontaneous. These spontaneous onset patients were also chosen consecutively from the hospital list; the first suitable match that was found being used in each case. No match could be found for ten of the induced patients and they were excluded, leaving 200 matched pairs in the study.

---

* Present address: Royal Prince Alfred Hospital Medical Centre, 100, Carillon Avenue, Newtown NSW, Australia 2042.

Spontaneous onset of labour was defined as the natural occurrence of regular painful uterine contractions leading to progressive dilatation of the cervix. Induction of labour was defined as a procedure initiating labour in a woman whose membranes were intact and who had no regular uterine contractions.

All the women selected had a singleton, cephalic presentation, with gestation between 37 and 41 weeks (i.e. not more than 21 days before the expected date of delivery, and not more than 13 days past it). Gestation was estimated from the date of the last menstrual period and if this was uncertain, by ultrasound measurement of the biparietal diameter. Any woman with a medical or obstetric complication during pregnancy was excluded. In addition, none of the women selected had had a previous Caesarean section, pre-term delivery, perinatal death, more than one abortion or treatment for infertility (Table I).

This selective sample was chosen in order to eliminate as many factors as possible that might not occur equally in the two groups and which would confound comparisons of the outcome of labour, made between them.

The reasons for induction recorded in the notes are shown in Table II. From the notes of the 400 women in the study, we recorded details of the antenatal care, labour, delivery and state of the newborn. Each set of notes was studied independently by two of us (P.Y. and A.F.) and the recordings double-checked.

Where there had been fetal monitoring during labour, each trace was assessed for the occurrence of fetal heart rate changes. These were defined as a fetal heart rate of less than 120 or more than 160 beats/minute, for more than two minutes, at any time during the labour.

The statistical tests used were chi-square and Fisher's exact test for the comparison of proportions and Student's t-test for the comparison of means.

TABLE I

Complications excluded from the sample

Medical problems during pregnancy

1. Blood pressure at any stage > 140 mm Hg systolic or > 90 mm Hg diastolic.
2. Proteinuria > trace at any stage, or trace on more than one occasion.
3. Abnormal glucose tolerance test result, or glycosuria > trace at any stage or trace on more than one occasion.
4. Long term drug treatment of any kind, e.g. for epilepsy.
5. Urinary tract infection in pregnancy or recurrent cystitis previously.
6. Haemoglobin of < 10 g/dl in pregnancy.
7. Any serious medical problem, e.g. deep vein thrombosis, cholestasis of pregnancy.

Obstetric problems diagnosed antenatally

1. Multiple pregnancy.
2. Breech presentation.
3. Completely uncertain dates.
4. Threatened miscarriage or antepartum haemorrhage during pregnancy.
5. Episode of threatened pre-term labour.
6. Hydramnios or oligohydramnios.
7. Rhesus isoimmunization.
8. Fetal growth retardation.
9. Fetal malformation.
10. Cephalopelvic disproportion.

Previous medical or obstetric problems

1. Deep vein thrombosis or pulmonary embolus.
2. Caesarean section.
3. Perinatal death.
4. Pre-term delivery.
5. More than one abortion.
6. Treatment for infertility.

TABLE II

Reasons for induction and gestation at induction

	Gestation (weeks)					No. of patients
	37	38	39	40	41	
Prolonged pregnancy, term	1	1	3	47	59	111
Social and other reasons of choice	0	7	20	8	0	35
Poor maternal weight gain	0	6	15	11	1	33
Mild hypertension	0	2	3	4	0	9
Previous rapid or difficult labour	0	2	5	1	0	8
False labour	0	1	1	2	0	4
Total	1	19	47	73	60	200

RESULTS

Description of sample

The breakdown by age, parity and social class of the two groups is shown in Table III. As age was matched to within four years either way, the breakdown into five-year bands is not identical in the two groups. Most of the patients were aged between 20 and 29; about half had one previous livebirth, and just over one-third were primiparae. Thirty-three women in each group had had a previous abortion.

There was no difference in maternal height (mean±SD) between the two groups: the height was 162·5±6·7 cm for the induced group and 162·2±5·6 cm for the spontaneous onset group.

There was also no difference in smoking habits. Seventy-six per cent of the women in both groups were non-smokers, and the number of cigarettes smoked per day (mean±SD) by the rest was 12·1±7·5 for the induced group and 13·5±8·8 for the spontaneous onset group.

There was a difference in gestational age between the two groups (Table III). More of the induced women were of 41 weeks gestation and fewer were of less than 40 weeks gestation. However, 93 per cent of the matched pairs differed in gestational age by 20 days or less, and 68·5 per cent of the pairs differed in gestational age by only 13 days or less.

Methods of induction used

Nearly all (183) of the induced patients were induced by means of artificial rupture of the membranes (ARM) accompanied by oxytocin infusion. In seven of these patients the oxytocin infusion preceded membrane rupture. Oxytocin infusion followed within 15 minutes of ARM in 95 patients, and within an hour in a further 57 patients. More than an hour elapsed between ARM and oxytocin infusion in only 24 patients. Fifteen had ARM alone, and the remaining two oxytocin infusion alone. Nine were treated with extra-amniotic prostaglandins before induction because of a markedly unfavourable cervix (Calder *et al*, 1977). None of these patients went into labour before membrane rupture.

Of the spontaneous onset patients, 46 had ARM before 5 cm dilatation. Eleven were augmented by oxytocin infusion alone and seven underwent both ARM and oxytocin infusion. The remaining 136 had no augmentation.

Length of labour

The first stage of labour was on average a little shorter in the induced than the spontaneous onset group ($p < 0{\cdot}05$). The length of the first stage (mean±SD) was 5·4±3·3 hours, for the induced group and 6·2±3·6 hours for the spontaneous onset group. Within the

TABLE III

Age, parity, social class and gestation

Percentages

Age	<20	20–24	25–29	30–34	35+	Total
Induced	5·0	32·5	48·5	11·5	2·5	100·0
Spontaneous onset	3·5	36·0	45·5	13·5	1·5	100·0
Parity	**0**	**1**	**2**	**3**	**4 or 5**	**Total**
Induced and spontaneous onset	37·5	51·5	8·5	1·5	1·0	100·0
Social Class	**I, II**	**III**	**IV, V**	**VIII***		**Total**
Induced and spontaneous onset	38·5	43·5	14·5	3·5		100·0
Gestation† (weeks)	**37**	**38**	**39**	**40**	**41**	**Total**
Induced	0·5	9·5	23·5	36·5	30·0	100·0
Spontaneous onset	4·0	15·0	32·5	30·0	18·5	100·0

* Social class VIII: armed forces

† Significant difference between induced and spontaneous onset groups $p < 0{\cdot}01$.

spontaneous onset group those women whose labours were augmented by oxytocin had a much longer first stage ($10\cdot5 \pm 4\cdot4$ hours). Excluding these women, the length of the first stage for the spontaneous onset group was $5\cdot7 \pm 3\cdot1$ hours. This is not significantly different from the induced group.

The second stage of labour was longer in the induced group than the spontaneous onset group ($p < 0\cdot01$). The length of the second stage was $0\cdot48 \pm 0\cdot40$ hours in the induced group and $0\cdot40 \pm 0\cdot30$ hours in the spontaneous onset group. However, when women receiving epidural anaesthesia were excluded, there was no longer a significant difference in the length of the second stage between the two groups. The length of the second stage was then $0\cdot34 \pm 0\cdot25$ hours for the induced group and $0\cdot31 \pm 0\cdot20$ hours for the spontaneous onset group.

Pain relief and delivery

There was a profound difference in the type of delivery experienced by women in the two groups (Table IV). While 99 per cent of the spontaneous onset group delivered spontaneously, only 67·5 per cent of the induced group did so. Thirty per cent of the induced group had forceps deliveries and 2·5 per cent had Caesarean sections. In the spontaneous onset group, 1 per cent had forceps deliveries and there were no Caesarean sections.

The Caesarean sections in the induced group were for fetal distress (three patients), prolonged labour (one patient) and cord prolapse (one patient).

Women in the induced group also received more pain relief than those in the spontaneous onset group. Forty-nine per cent had epidural anaesthesia compared with 13·5 per cent of the spontaneous onset group. Only 6 per cent of the induced group required no pain relief or nitrous oxide/oxygen only, compared with 31·5 per cent of the spontaneous onset group.

Within the induced group, those women given epidurals were most likely to have forceps deliveries. However, the excess of operative deliveries in this group was not only due to an excess of epidurals. Women who were induced had a significantly higher proportion of operative deliveries than those with spontaneous onset of labour, whether they had epidurals or whether they did not ($p < 0\cdot001$ in both cases).

Not only did the induced women receive more pain relief, they were also more often monitored (Table V). The alternative to monitoring was auscultation every 15 minutes in the first stage and every 5 minutes in the second stage. Thus it is not surprising that fetal heart rate changes were more often recorded in the monitored patients, who were observed continuously. One might therefore expect more operative deliveries in monitored patients for this reason alone.

TABLE IV

Pain relief and type of delivery

	None or Entonox only	Pethidine only	Epidural		Total
			With pethidine	Without pethidine	
Induced group					
Spontaneous	11	76	13	35	135
Forceps	0	12	13	35	60
Caesarean	1	2	0	2	5
Total	12	90	26	72	200
Spontaneous onset group					
Spontaneous	63	109	11	15	198
Forceps	0	1	1	0	2
Caesarean	0	0	0	0	0
Total	63	110	12	15	200

TABLE V

Fetal monitoring and heart rate changes

	Induced		Spontaneous onset		Total	
	Monitored	Not monitored	Monitored	Not monitored	Monitored	Not monitored
Heart rate changes	67	5	11	5	78	10
No heart rate changes	63	64	27	155	90	219
Total	130	69	38	160	168	229

Note: Monitoring not known for one induced case and two spontaneous onset cases.

TABLE VI

Operative deliveries by epidural anaesthesia and monitoring

	All gestations		Gestation $\leq$40 weeks	
	Induced	Spontaneous onset	Induced	Spontaneous onset
No epidural, not monitored				
Number	39	145	21	122
Operative deliveries	0	0	0	0
Per cent of primiparae	28	32	19	32
No epidural, monitored				
Number	63	27	46	18
Operative deliveries*	15‡	1	12‡	0
Per cent of primiparae	30	59	33	50
Epidural, not monitored				
Number	30	15	23	12
Operative deliveries	13§	0	10§	0
Per cent of primiparae	30	33	30	25
Epidural, monitored				
Number	67	11	49	9
Operative deliveries†	36§	1	23§	0
Per cent of primiparae	52	73	45	67

Note: Monitoring not known for one induced and two spontaneous onset cases.
* Including 3 Caesarean sections in induced group
† Including 2 Caesarean sections in induced group
Significant difference between induced and spontaneous onset groups: ‡ $p < 0{\cdot}05$ § $p < 0{\cdot}025$

Consequently, the combination of more frequent epidurals and more frequent monitoring in the induced group might have accounted for the excess of operative deliveries in this group.

The rates of operative deliveries in women grouped according to their epidural/monitoring experience are shown in Table VI. Figures are given both for the whole sample and for women of 40 weeks gestation or less. Among those women who did not receive epidural anaesthesia or fetal monitoring, no operative deliveries occurred in either the induced or spontaneous onset series. In all other sub-groups there were significantly more operative deliveries in the induced series. The excess of operative deliveries in the induced women cannot therefore be accounted for by the pain relief and monitoring they received. Except for those women receiving neither epidural anaesthesia nor monitoring, membership of the induced group itself appears to carry an added risk of an operative delivery. This risk is apparent even when women of over 40 weeks gestation are excluded from the analysis.

It is notable that none of the 18 spontaneous onset women whose labours were augmented by oxytocin had operative deliveries. Thirteen of these women were monitored, eight had epidurals.

Although the rate of operative deliveries was higher for induced women of all parities, the most striking difference in rates occurred in primiparae. For instance, among induced primiparae with epidurals, an operative delivery occurred in 35 out of 44 patients. In the comparable spontaneous onset group, there was one forceps delivery in 13 patients ($p < 0{\cdot}001$). However, the significant excess of operative deliveries in the induced series was not due to an excess of primiparae. None of the induced sub-groups contained a higher proportion of primiparae than the comparable spontaneous onset sub-group; in fact the reverse was the case (Table VI).

The forceps deliveries were examined further to discover how many were easy 'lift outs' and how many were 'more difficult' forceps deliveries, i.e. either mid-cavity or involving moderate or strong traction (Table VII). The incidence of 'more difficult' forceps deliveries was higher in the induced series, but the differences within each sub-group were not significant at the 0·05 level. Rotation was necessary in about two-thirds of the 'more difficult' forceps deliveries in the induced group. No rotation was needed in the two cases in the spontaneous onset group.

TABLE VII

More difficult forceps deliveries, rotations and fetal heart rate changes by epidural/monitoring groups*

	Induced	Spontaneous onset
No epidural, not monitored		
Number	39	145
More difficult forceps	0	0
Heart rate changes	1	4
No epirudal, monitored		
Number	63	27
More difficult forceps	6	1
Rotations	4	0
Heart rate changes	33	8
Epidural, not monitored		
Number	30	15
More difficult forceps	7	0
Rotations	5	0
Heart rate changes	4	1
Epidural, monitored		
Number	67	11
More difficult forceps	21	1
Rotations	14	0
Heart rate changes	34	3

* Number of rotations performed in the more difficult forceps deliveries.

If induction itself does carry an extra risk of an operative delivery, then this should also be apparent amongst those women who had neither epidural anaesthesia nor monitoring during labour. However, no operative deliveries occurred in this sub-group, and it was therefore examined to see whether it included women who in some measurable way, could be said to be more 'ready' for labour than women in the other epidural/monitoring sub-groups. Compared to the rest of the induced series, this sub-group contained a higher proportion of women of 41 weeks gestation (46 per cent compared to 26 per cent; $p < 0{\cdot}05$) and a slightly higher proportion of multiparae (72 per cent compared to 61 per cent). In addition, the length of the first stage (mean ± SD) in this sub-group was shorter than that in any other sub-group, being $3{\cdot}80 \pm 1{\cdot}95$ hours compared with $5{\cdot}72 \pm 3{\cdot}29$ hours in the rest of the induced group ($p < 0{\cdot}001$). However, no differences between this sub-group and the rest could be found in the time taken from induction to the start of labour, or in the Bishop score (Bishop, 1964). Since the components of the Bishop score were not always completely recorded in the notes, and as we are aware that the assessment of Bishop score tends to vary between different obstetricians, women in this sub-group may in fact have had more favourable cervices, even though this was not apparent in our data.

Rather more of the induced patients had a postpartum loss of 500 ml or more (17 compared with 7 in the spontaneous onset series; $p > 0{\cdot}05$). An episiotomy was performed on about the same number in each group: 137 in the induced group and 121 in the spontaneous onset group. The incidence of postpartum haemorrhage was slightly higher in those who received episiotomies than in those who did not: 7 per cent compared to 3 per cent ($p > 0 \cdot 05$).

State of the fetus and newborn

The incidence of fetal heart rate changes in epidural/monitoring sub-groups is shown in Table VII. Except for the sub-group without epidural or monitoring, the incidence of heart rate changes was slightly higher in the induced series, but the differences were not significant at the 0·05 level. Moreover, there was no evidence that epidural anaesthesia increased the incidence of fetal heart rate changes, once monitoring was held constant. It should be stressed that our definition of fetal heart rate change was unsophisticated (see Methods).

All the babies were liveborn and there were no neonatal deaths.

There was no difference in birth weights between the two groups. The birth weight for boys (mean±SD) was $3 \cdot 46 \pm 0 \cdot 45$ kg in the induced group and $3 \cdot 41 \pm 0 \cdot 35$ kg in the spontaneous onset group. For girls the comparable figures were $3 \cdot 29 \pm 0 \cdot 39$ kg and $3 \cdot 30 \pm 0 \cdot 40$. One baby in the induced group and two in the spontaneous onset group weighed less than 2·5 kg at birth. All three were born in good condition.

Considerably more of the induced babies needed resuscitation by endotracheal intubation: 15 compared to 4 in the spontaneous onset group ($p < 0 \cdot 025$).

Of the babies requiring resuscitation in the induced group, two were delivered by Caesarean section and five were delivered within two hours of pethidine administration. Five were delivered with forceps; the remaining eight were delivered spontaneously. In the spontaneous onset group, one of the four intubated babies was delivered within two hours of pethidine being given. All were delivered spontaneously.

Apgar scores at one minute were incompletely recorded for 35 (17·5 per cent) of the induced babies and 38 (19 per cent) of the spontaneous onset babies. It appeared that babies in poor condition were less likely to have Apgar scores recorded than those in good condition. Because of this, we considered it misleading to compare the distribution of Apgar scores in the two groups.

Nine babies in the induced group, compared to four in the spontaneous onset group, were sent to the Special Care Baby Unit ($p > 0 \cdot 05$). In the induced group the indications were: respiratory distress syndrome (three babies); birth asphyxia (two babies); hypothermia (two babies); low dextrostix reading (one baby); and a cyanotic turn (one baby). One of the babies with respiratory distress syndrome was assessed as being at 36 weeks gestation (weight 2·7 kg) though gestation was thought to be 39 weeks at induction. In the spontaneous onset group, the reasons for admission to Special Care were: respiratory distress syndrome (two babies); birth asphyxia (one baby); and jaundice (one baby). Most of the babies were kept in Special Care for one or two days only, and all were discharged home within 15 days.

Rather more of the induced babies developed jaundice; 77 compared with 59 in the spontaneous onset group ($p > 0 \cdot 05$). Of those developing jaundice, 60 (78 per cent) in the induced group and 48 (81 per cent) in the spontaneous onset group had their bilirubin levels measured. There was no significant difference in the bilirubin levels (mean±SD) of the two groups: $11 \cdot 5 \pm 3 \cdot 13$ mg per cent for the induced group and $10 \cdot 40 \pm 3 \cdot 31$ mg per cent for the spontaneous onset group.

There was no difference between the two groups of women in their ability to breast feed their babies. A slightly higher proportion of spontaneous onset mothers stated an intention to breast feed at their first antenatal visit (67 per cent compared to 59·5 per cent, $p > 0 \cdot 05$). Of those intending to breast feed, the same proportion in each group were actually doing so on discharge: 86 per cent in the spontaneous onset group and 82 per cent in the induced group. 'Discharge' here does not necessarily mean the day on which the women left hospital, but rather the day when records stop. In many cases, the woman was followed-up by the midwife after returning home.

DISCUSSION

Our study finds a contrast in the experiences of those women who had labour induced and those who started labour spontaneously. Our group of spontaneous onset women had a remarkably good record of labour and delivery. Their mean length of labour was 6·6 hours and only 2 out of 200 failed to deliver spontaneously: a very low percentage compared to that found in other recent studies (Cartwright, 1977; Martin *et al*, 1978). Our findings are that healthy women, with no medical or obstetric abnormalities, who are allowed to come into labour spontaneously, will nearly all experience an uncomplicated labour and delivery. Further, even if these women receive intervention during labour, in the form of oxytocin infusion, epidural anaesthesia or fetal monitoring, an operative delivery can normally be avoided.

Women in the induced group, who also had no medical or obstetric complications, not only received more intervention during labour, but were also more likely to have a forceps delivery or a Caesarean section. This increased risk of an operative delivery was not fully explained by the extra pain relief and monitoring received.

There appear to be two possible explanations for these findings. It could be that the high rate of forceps deliveries among the induced women reflects not so much the inability of these women to deliver spontaneously, but rather the close attention of the supervising obstetrician. Having started a woman's labour electively and closely followed it through to full dilatation with fetal monitoring, the obstetrician may well feel that he can ensure a successful delivery by intervening in the second stage as well. If such a bias in attitude does exist among obstetricians, one would be more likely to detect it in a retrospective study than in a prospective one, since a prospective study would be carefully designed to eliminate such bias.

The second possible explanation is that an induced woman really is more likely to need an operative delivery. Most of the forceps deliveries that occurred were for fetal distress or delay in the second stage, while the Caesarean sections were for fetal distress, prolonged labour and cord prolapse. One would therefore have to postulate that induced labour tends to produce these problems. The mechanism by which this might occur can only be guessed at; to investigate it would need a different sort of study from the present one.

In contrast to the general picture, we found one group of induced women who received neither epidural anaesthesia nor fetal monitoring and who all delivered spontaneously. This group differed from the rest of the induced women in two ways: more of them were beyond 40 weeks gestation and on average they had more rapid labours. It is possible that this group were very close to spontaneous labour and that physiological changes may have occurred which protect against some of the troublesome aspects of induction. In addition, it is likely that these women who, early in labour, appeared to be progressing very well, would have avoided being monitored or given an epidural. It might be argued that such women are the ideal candidates for elective induction and that means of identifying them, for example by the state of the cervix and the sensitivity of uterine contractility to a small dose of oxytocin (Turnbull and Anderson, 1968*a* and *b*) should be used before induction takes place.

The babies born after an induced labour appeared to be in rather worse condition than those born after spontaneous onset, in that considerably more of them needed resuscitation by intubation. The additional intervention experienced by the induced woman does therefore appear to result in increased morbidity for the baby, measurable even in a relatively small group of 200 births.

What is less clear however, is whether any benefits can be gained from inducing labour at term even in the absence of medical or obstetric complications, rather than awaiting the spontaneous onset of labour. Some studies have expressed the view that a high induction rate confers no particular benefit (O'Driscoll *et al*, 1975; Chalmers *et al*, 1976*a* and *b*). Others have argued that elective induction leads directly to a fall in perinatal mortality (McNay *et al*, 1977).

In such a climate, there is inevitably a diversity of obstetric practice. Further, it is not always the obstetrician who advocates induction. Women themselves often ask to be induced for various reasons; for instance to avoid prolonged pregnancy or to simplify their domestic arrangements.

Our study suggests that induction may entail certain disadvantages. In a retrospective study, it is not possible to explain why these disadvantages occurred. But this uncertainty should not detract from the significance of the findings: compared to labour after spontaneous onset, induced labour was more often accompanied by other procedures and carried an increased risk of an operative delivery.

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THE TECHNIQUE OF FETAL ELECTROENCEPHALOGRAPHY DURING LABOUR

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Summary

A method for recording the fetal electroencephalogram (EEG) during labour is described. Technical specifications for the construction of a suitable electrode are detailed. One hundred and twenty-three patients in labour were monitored using this technique and the success rate of obtaining records for analysis was 91 per cent.

MYERS (1972) found that when pregnant monkeys at term were subjected to episodes of partial asphyxia the fetuses developed lesions of the cerebral hemispheres. In the mature human fetus and newborn, Towbin (1970) found that one of the most frequent sites of hypoxic damage was the cerebral cortex. The fetal cerebral hemispheres at term seem very sensitive to hypoxic damage. For recording the fetal electroencephalogram (EEG) two electrodes were used, one applied to each parietal area.

A programme of recording the fetal EEG during labour was commenced at Harare Hospital, Salisbury, in June, 1974, and we now report on the technical aspects of obtaining such recordings.

METHODS

The electrode

The electrode we used (Fig. 1) was a modification of the one described by Rosen and Scibetta (1969). The total diameter of the electrode unit was 25 mm. The electrode itself was a silver pin, 2 mm in diameter, 8 mm in length and with a 20 degree inclusive angle, machined on the pin point. It was set with 'Araldite' into the centre of an 18 mm diameter hemispherical perspex cup so that its tip lay 1 mm below the edge of the cup. The perspex cup had circular grooves to enable the fetal scalp to be sucked up onto the recording electrode point without obstructing the suction lead. A 3 mm diameter cannula tubing was moulded into the lateral aspect of the perspex cup and a suction pressure of 20 cm water was applied via the tubing to maintain contact between the electrode and the fetal scalp. A smooth outer Silicone rubber mould was mounted over the perspex cup and around the suction tubing.

The internal wire, soldered onto the silver electrode, was connected to a selected channel on the EEG headboard. A single screened cable of commercial microphone quality was used and the outer screen was earthed at the ground terminal of the EEG headboard (Figs. 2 and 3). The construction of the outer mould was such that a 1 mm space existed between the outer edge of the perspex cup and the rubber casing. This enabled the outer casing to be peeled back

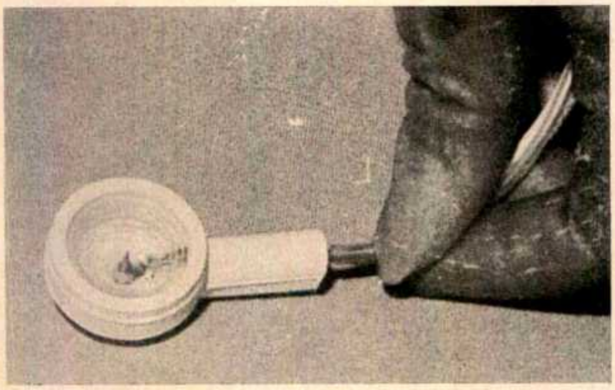

FIG. 1
The fetal EEG electrode.

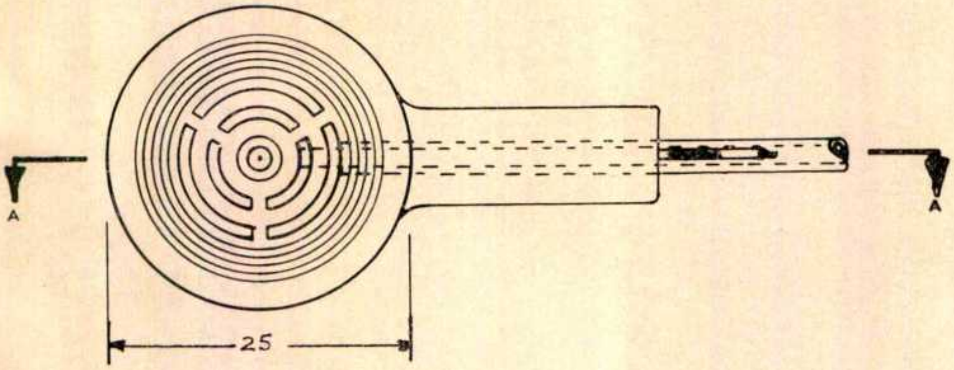

FIG. 2
Inner surface view of fetal EEG electrode drawn to scale.

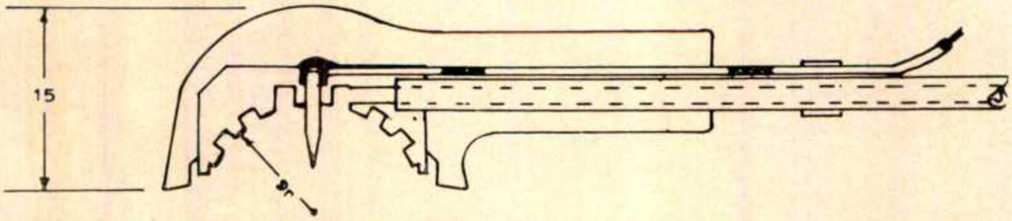

FIG. 3
Sectional view of fetal EEG electrode drawn to scale.

off the perspex cup for cleaning and sterilizing procedures.

A separate spiral electrode was used to record the fetal heart rate.

Monitoring technique

A room near the labour ward was set aside. To screen out electrical interference from the mains electrical supply, a sheet of fine copper wire mesh was laid on the floor and earthed to a point outside the room. Matting was placed on top of this. Patients in the active phase of labour were selected for monitoring, after informed consent was obtained. They were nursed in the left lateral position on a metal bed with a rubber mattress. The progress of labour was charted on the partogram described by Philpott (1972).

An intravenous infusion of 5 per cent dextrose was commenced and a continuous lumbar epidural block was inserted. A vaginal examination was made and a fetal scalp blood sample was obtained for pH measurement.

The electrodes, which had been sterilized for 24 hours in hibitane 1:2000, were than applied, one after another to the parietal eminences, under full aseptic technique with the patient in the lithotomy, or left lateral position. The distance between electrodes was always more than 4 cm (Rosen and Scibetta, 1969). A hibitane spray was used as lubricant as Dettol cream caused electrical interference. A negative pressure of 20 cm of water was maintained throughout labour. The electrode was massaged onto the fetal scalp for a few seconds in order to drive the electrode pin into the superficial layers of the fetal scalp.

A spiral electrode for fetal heart rate monitoring was then inserted into the fetal scalp. The patient was catheterized, turned into a lateral position and an abdominal pressure transducer was applied.

The fetal EEG trace was recorded on a Standard 8 channel portable Elema Schonander Recorder. The paper speed was 30 mm per second, the time constant was 0·30 seconds or 0·15 seconds, the filters were set at FHz30 and the sensitivity at 50 µv/cm. The fetal heart rate and uterine contractions were monitored by an external abdominal transducer at a paper speed of 1 cm per minute on a Hewlett-Packard cardiotocograph.

Two observers were necessary: one to run the EEG machine and mark events occurring in labour upon the trace and one to care for the patient from an obstetric point of view.

RESULTS

A total of 123 patients was monitored, satisfactory recordings being obtained from 112 (91 per cent). None of the infants had significant scalp damage. The major problem encountered was ECG artifact on the EEG trace. It occurred if amnion or amniotic fluid was trapped between the EEG electrode and the scalp and it is thus essential to rupture membranes and clean the scalp field before EEG electrode application. It could also be due to blockage of the suction tubing, in which case the EEG electrode had to be removed, cleaned and reapplied. In some cases bluntness of the EEG electrode prevented its penetration into scalp tissue.

Once the electrodes were correctly applied and a good EEG tracing was being obtained, they continued to function undisturbed by vaginal examinations and scalp blood sampling until the head crowned. The pressure of the uterine contractions tended to improve contact between fetal scalp and parietal EEG electrodes.

The routine was simple and electrode placement was easily learnt by doctors and midwives.

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THE EFFECT OF FETAL HEAD COMPRESSION AND FETAL ACIDAEMIA DURING LABOUR ON HUMAN FETAL CEREBRAL FUNCTION AS MEASURED BY THE FETAL ELECTROENCEPHALOGRAM

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Summary

We investigated the effect of head compression and acidaemia during labour in 25 African primigravidae. Evidence that head compression had occurred during labour was confirmed by both clinical and radiological means at the end of a trial of labour. No significant difference could be demonstrated in the quantity of fetal electroencephalogram (EEG) abnormality that occurred in the groups with marked head compression as compared to the groups without marked head compression. Deterioration in the fetal EEG to a flat record known as electrocerebral silence (ECS) was associated with the development of acidaemia. As fetal heart rate (FHR) decelerations appeared the percentage of ECS in the fetal EEG record increased significantly ($P < 0 \cdot 05$), and likewise, as fetal acidaemia developed a highly significant increase in ECS in the fetal EEG was demonstrated ($P < 0 \cdot 001$). We concluded that in the management of trial of labour there was no significant deleterious change in the fetal EEG as a result of head compression, unless fetal acidaemia supervened. However, in the majority of these cases a significant increase in ECS to more than 20 per cent occurred in association with only moderate acidaemia (pH 7·25 to 7·30).

CEPHALOPELVIC disproportion remains a major obstetrical hazard in developing countries and contributes significantly to perinatal death rates. In many cases disproportion and fetal asphyxia co-exist and make the exact cause of death difficult to determine. Perinatal statistics from Harare Hospital, Salisbury reveal that between 15 to 20 per cent of perinatal deaths are due to disproportion and its sequelae. Little is known of the long term effects in survivors, but Axton and Levy (1974) reviewed the histories of 212 mentally retarded Rhodesian African children and found a definite history of birth trauma in 32·7 per cent of those with an acquired perinatal defect. Brown *et al* (1974) selected 93 asphyxiated newborn infants with abnormal behaviour patterns and found that 32 (or 34 per cent) were asphyxiated as a result of birth injury. On neurological follow up of this group only 55 per cent were subsequently normal.

Monitoring fetal cerebral function by recording the fetal electroencephalogram (EEG) during labour has been done for over two years in this obstetric unit.

The aim of the present study was to record the fetal EEG during labour in a series of high-risk African primigravidae, to determine the effect of head compression and acidaemia on the electrical activity of the fetal brain.

METHOD

Twenty-five African primigravidae in early labour were selected and informed consent was obtained for fetal monitoring. The patients were all of high-risk, four had oxytocin inductions of labour for medical reasons and 21 patients had a spontaneous onset of labour. All infants were 36 weeks or more by gestational age and all were of appropriate size for gestational age, being above the 10th centile (Lubchenco *et al*, 1963). The technique of monitoring the fetal EEG has been described (Wilson *et al*, 1979).

When the active phase of labour commenced, the patient received an intravenous dextrose infusion and a continuous lumbar epidural block. A pelvic assessment was made with particular reference to the height of the fetal head in fifths above the pelvic brim, the degree of moulding out of a score of 6 as described by Philpott and Stewart (1974), the position of the fetal head and the dilatation of the cervix. The progress of labour was recorded on the composite labour graph (Philpott, 1972). The same observer made vaginal assessments four-hourly or more often when delay in progress became evident. If there was delay in the progress of labour, a vaginal examination was made and in the absence of signs of gross disproportion, malpresentation or significant fetal distress, labour was augmented by oxytocin for a period not exceeding six hours. If, during this period, there was fetal or maternal distress a Caesarean section was performed. Scalp blood pH measurements were made at the start of monitoring, at the end of the first stage of labour and up to five times in the intervening period. The blood sample was collected with the patient in the left lateral position and the same investigator and technician performed the collection and analysis on a PHM 71 Mark II Acid-Base Analyser. A result was accepted if two pH readings on the same sample were less than 0·02 units apart.

The fetal heart rate and uterine contractions were monitored on a Hewlett Packard cardiotocograph and the fetal EEG was recorded on an eight channel portable Elena Schonander Recorder as continuously as possible as full dilatation approached or delay in the progress of labour became evident. At the end of the first stage of labour a second pelvic assessment was made in order to assess the presence or absence of cephalo-pelvic disproportion.

The infants had Apgar scores, routine perinatal care and an EEG within 30 minutes of birth. The biparietal diameter was measured with calipers at birth and as soon as possible after delivery each mother had an X-ray pelvimetry. Clinical decisions were taken by the consultant in charge of the obstetric team on duty and not by the research team.

Analysis of the data

Only changes that occurred during the final hour of monitoring were analysed. In patients who had a vaginal delivery this represented the hour before full dilatation of the cervix and in those who had a Caesarean section the hour before the decision to deliver abdominally was made.

Fetal EEG abnormality

The end point of fetal EEG deterioration as established by this series, the work of Rosen (1967) on the fetal guinea pig and Mann *et al* (1970, 1971 and 1972) on the fetal lamb, is a trace in which there is loss of all wave frequencies and an amplitude of under 2 μv. Previously described as 'iso-electric' or 'flat', the American Electroencephalographic Society (1976) has recommended that this change in the EEG be known as electrocerebral silence (ECS). The fetal EEG recorded during the time specified was analysed to determine the total duration of ECS and this was expressed as a percentage of the record obtained for that period.

Each patient thus had a percentage of ECS for the final hour of monitoring and this has been taken to be an index of fetal cerebral abnormality. The percentage of ECS for each patient is shown in Table I.

Fetal acidosis

The fetus was considered to be 'at risk' of acidosis if: (i) the scalp pH at the end of monitoring was 7·25 or less; (ii) the scalp pH had fallen by 0·10 units or more during the course of labour; (iii) the scalp pH had fallen by 0·05 units or more between the last two intrapartum measurements which were in the order of an hour apart.

The fetus was considered acidotic if the scalp

TABLE I

Data on the clinical features used to identify the group of fetuses with severe head compression during labour

Series No.	Head moulding (maximum score = 6)	Head level (in fifths above the brim)	Delivery outcome	Cervical dilatation (cm)	Fetal heart rate pattern	Final scalp blood pH	ECS (per cent of recording period)
Group I—Fetuses without severe head compression							
1	5	2	SVD	10	Normal	7·34	1·5
2	5	3	VE	10	Normal	7·34	1·5
3	4	3	SVD	10	Early decelerations v	7·31	4·6
4	5	1	SVD	10	Normal	7·34	3·5
5	5	3	SVD	10	Normal	7·46	3·5
6	3	2	VE	10	Normal	7·35	1·5
7	5	3	VE	10	Normal	7·34	2·0
8	6	2	SVD	10	Normal	7·30	0
9	5	3	VE	10	Normal	7·35	0
11	4	3	VE	10	Variable decelerations	7·28	23·0
12	5	2	SVD	10	Late decelerations	7·30	32·0
16	1	4	LSCS	7	Late decelerations t	7·21*	100·0
21	0	3	VE	10	Late decelerations	7·19*	37·0
22	3	3	VE	10	Late decelerations	7·26*	100·0
24	1	1	SVD	10	Variable decelerations	7·34	12·0
25	5	3	VE	10	Late decelerations	7·32	1·0
Mean values						7·31	20·19
Group II—Fetuses with severe head compression							
10	0	5	LSCS	5	Late decelerations	7·26*	1·3
13	5	5	LSCS	4	Early decelerations tv	7·27*	100·0
14	6	4	LSCS	10	Late decelerations t	7·23*	46·0
15	5	4	LSCS	4	Late decelerations t	7·38	0·0
17	5	5	LSCS	4	Early/late decelerations t	7·30	0·8
18	4	3	LSCS	9	Late decelerations t	7·26*	52·0
19	4	5	LSCS	3	Late decelerations t	7·26*	25·0
20	5	3	LSCS	8	Late decelerations v	7·30	16·0
23	5	4	LSCS	10	Late decelerations v	7·32	10·0
Mean values							27·90

ECS = electrocerebral silence
SVD = spontaneous vaginal delivery
VE = vacuum extraction
LSCS = lower segment Caesarean section

t = baseline tachycardia
v = loss of beat-to-beat variability
* = low or falling pH

pH at any point during labour was 7·20 or less.

The final scalp pH values are shown in Table I and the eight fetuses with low or falling pH values are indicated by an asterisk.

Fetal heart rate (FHR) abnormality

The FHR was classified as abnormal if decelerations of an early, late or variable nature were identified on the cardiotocograph trace during the final hour of monitoring. Seventeen patients had decelerations of the FHR and in ten of these, baseline abnormalities and loss of beat-to-beat variability was present as well. The data is shown in Tables I and IV.

Diagnosis of cephalopelvic disproportion and head compression

Cephalopelvic disproportion was diagnosed by retrospective appraisal of the clinical features and by radiographic studies using prediction charts for the plane of the pelvic brim (Chassar-Moir, 1971).

Clinical features assessed for the retrospective diagnosis of cephalopelvic disproportion in-

TABLE II

Evidence of cephalopelvic disproportion at the brim plane using radiological prediction charts (Chassar-Moir 1971)

Series No.	Brim measurements (cm) AP	Brim measurements (cm) Available transverse	Biparietal diameter at birth (cm)	Brim disproportion	ECS (per cent)
Group A—No brim disproportion					
1	11·8	14·5	10	No	1·5
2	11·5	12·5	10	No	1·5
4	12·0	12·2	10	No	3·5
5	12·8	10·0	10	No	3·5
6	12·5	12·0	10	No	1·5
8	11·8	12·5	9	No	0·0
10	12·5	11·3	10	No	1·3
11	11·5	12·0	10	No	23·0
12	12·4	11·5	11	No	32·0
16	10·8	13·5	9	No	100·0
17	13·5	12·2	10	No	0·8
22	11·3	11·5	10	No	100·0
24	13·2	10·0	10	No	12·0
25	12·4	12·6	10	No	1·0
9	11·2	12·6	10	No	0·0
Mean					18·7
Group B—Brim disproportion					
3	10·4	11·0	10	Yes	4·6
7	10·2	12·4	11	Yes	2·0
13	10·8	11·0	10	Yes	100·0
14	9·2	10·4	9	Yes	46·0
15	11·0	11·4	10	Yes	0·0
18	11·6	11·0	10	Yes	52·0
19	10·8	11·0	11	Yes	25·0
20	10·2	10·5	10	Yes	16·0
21	10·5	12·0	10	Yes	37·0
23	11·3	11·0	11	Yes	10·0
Mean					29·3

ECS = electrocerebral silence
AP = anterior-posterior brim diameter

cluded: (i) delay in the progress of labour with failure to achieve full dilatation of the cervix despite oxytocin stimulation; (ii) increasing head moulding; and (iii) failure of the fetal head to descend during labour.

Two groups of patients were identified by this method. Group I consisted of 15 patients who had a vaginal delivery and one patient who required an abdominal delivery for fetal distress in the absence of other signs of disproportion. These 16 patients progressed normally during the active phase of labour and in 15, vaginal deliveries were achieved within 45 minutes of full dilatation of the cervix. These labours were efficient, progressive and unassociated with features of marked fetal head compression. Group II consisted of nine patients who required delivery by lower segment Caesarean section. Seven of these patients had evidence of delay in the progress of cervical dilatation and despite oxytocin augmentation of labour in five patients, full dilatation was not achieved. Two patients with high, well-moulded fetal heads were unsuitable for oxytocin stimulation as fetal distress was present at the time that delay was diagnosed. Two patients achieved full dilatation but operative delivery was indicated as the fetal heads were wedged at the pelvic brim and did not descend with pushing. Thus patients in Group II who were diagnosed as having head compression all had: (i) lower segment Caesarean sections for a failed trial of labour despite oxytocin stimulation in appropriate cases; (ii) marked moulding of the occipito-parietal and parieto-parietal suture lines; and (iii) failure of the fetal head to descend after a trial of labour.

The clinical data which enabled the patients to be divided into Groups I and II is shown in Table I.

Radiographic assessment of cephalopelvic disproportion

By the use of prediction charts for disproportion at the pelvic brim (Chassar-Moir, 1971), patients with radiographic evidence of cephalopelvic disproportion were diagnosed. Table II shows the data for the series.

Ten patients (Group B) had radiographic measurements diagnostic of cephalopelvic disproportion but three of these were not in the group diagnosed clinically and two, thought clinically to have disproportion, did not have supporting radiological evidence. Sixteen patients (Group A) did not have radiological evidence of cephalopelvic disproportion.

RESULTS

Non-parametric tests were used in the statistical analysis because the patient sample was small and certain parameters such as head moulding or level above the pelvic brim could only be assessed qualitatively. The percentage of ECS for each patient was compared to other fetal factors.

The effect of head compression on the fetal EEG

In the groups of patients identified by clinical assessment of head compression (Table I), the mean percentage of ECS in Group I was 20·19 and in Group II was 27·90. This difference was not significant (Mann Whitney; $P > 0\cdot05$).

In the two groups of patients identified by radiological assessment of cephalopelvic disproportion, the mean duration of ECS was 18·7 per cent in those patients with no disproportion and 29·3 per cent in those with disproportion.

There was no significant difference in the percentage of ECS in the records of those patients with radiological evidence of brim disproportion compared to those without brim disproportion (Mann Whitney; $P > 0\cdot05$).

The effect of acidosis on the fetal EEG

The percentage of ECS in the fetal EEG increased significantly as the fetal scalp pH at the end of the first stage of labour decreased (Spearman's Rank Correlation Coefficient $P < 0\cdot001$). A very definite relationship existed between the development of ECS in the fetal EEG during the final hour of the first stage of labour and the development of fetal acidosis at the end of the first stage of labour.

Figure 1 shows the relationship between the final scalp pH value and the percentage of ECS in the fetal EEG in the previous hour of monitoring. It can be seen that rapid deterioration in the fetal EEG occurred as the pH fell and even at 'pre-acidotic' levels (pH 7·20 to 7·25) marked changes were present with the cessation of electrical activity in the fetal brain.

Since the process leading to fetal acidaemia plays such an important role in fetal EEG

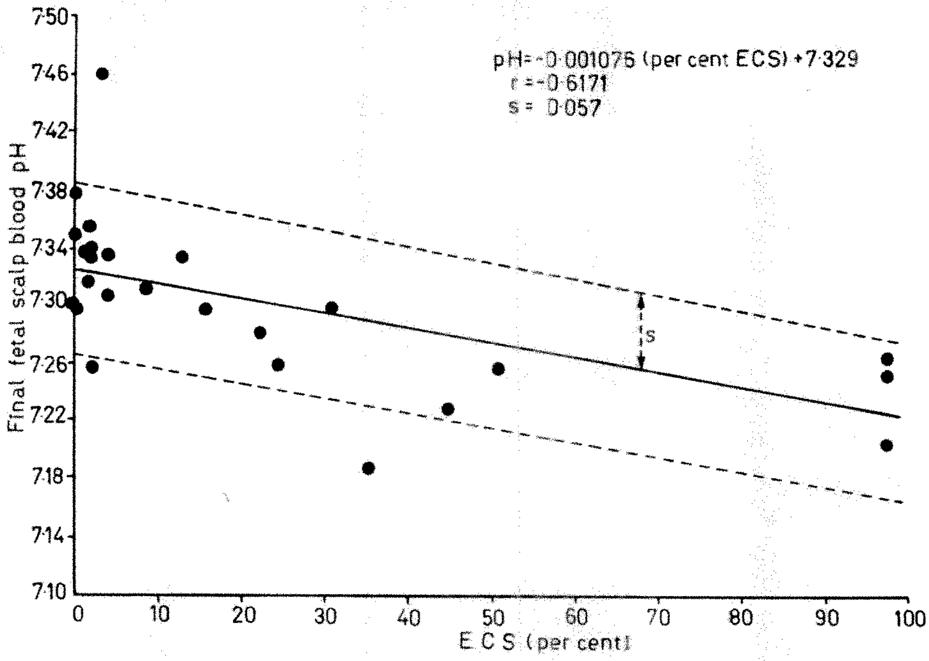

FIG. 1
The relationship between pH and electrocerebral silence (ECS).

TABLE III
The effect of head compression and fetal acidaemia on the percentage of electrocerebral silence (ECS) in the fetal EEG

	Head compression	No head compression	Total
No. acidotic	5	3	8
Mean ECS (per cent)	44·9	79·0	57·7
No. non-acidotic	4	13	17
Mean ECS (per cent)	6·7	6·6	6·7

deterioration, an attempt was made to separate out its influence in labours complicated by severe disproportion and head compression. Amongst the nine patients with clinical evidence of head compression, five were 'at risk' of acidosis and four were non-acidotic at the end of the trial of labour. The data are shown in Table III. The mean percentage of ECS in the fetal EEG records of fetuses with both head compression and acidosis was 44·9 per cent compared to a mean of 6·7 per cent in those with head compression but no acidosis. This difference was very significant (Mann Whitney Test; $0{\cdot}02 > P > 0{\cdot}01$). There was no statistical difference between the percentage of ECS in the fetal EEG of the fetuses who were not acidotic regardless of whether head compression was present or not.

Relation between FHR decelerations and fetal EEG

The percentage of ECS for each patient was compared to FHR decelerations. There was a statistically significant relationship between increasing percentages of ECS in the fetal EEG and the development of FHR deceleration patterns during labour (Biserial Correlation $P < 0{\cdot}05$).

There were 17 fetuses with FHR decelerations. The patients were grouped as follows: (1) those with normal FHR patterns and normal scalp blood pH values (Group A); (2) those with FHR decelerations and normal scalp blood pH values (Group B); (3) those with both FHR decelerations and scalp blood pH values indicating actual or potential acidosis (Group C).

Table IV shows the data with the percentage of ECS for each patient and the mean percentage for each group. The mean percentage of ECS

TABLE IV

The relationship between fetal EEG abnormality, FHR decelerations and low scalp-blood pH values

Series number	ECS (per cent)	Final scalp blood pH	Fetal heart rate pattern	
Group A: Normal FHR patterns and normal scalp pH values				
1	1·5	7·34	Normal	
2	1·5	7·34	Normal	
4	3·5	7·34	Normal	
5	3·5	7·46	Normal	
6	1·5	7·35	Normal	
7	2·0	7·34	Normal	
8	0	7·30	Normal	
9	0	7·35	Normal	
Mean	1·7	7·35		
Group B: Abnormal FHR patterns but normal scalp pH values				
3	4·6	7·31	Early decelerations	v
11	23·0	7·28	Variable decelerations	
12	32·0	7·30	Late decelerations	
15	0	7·38	Late decelerations	t
17	0·8	7·30	Early and late decelerations	t
20	16·0	7·30	Late decelerations	v
23	10·0	7·32	Late decelerations	v
24	12·0	7·34	Variable decelerations	
25	1·0	7·32	Late decelerations	
Mean	11·0	7·32		
Group C: Abnormal FHR patterns and abnormal scalp pH values				
10	1·3	7·26	Late decelerations	
13	100·0	7·27	Early decelerations	tv
14	46·0	7·23	Late decelerations	t
16	100·0	7·21	Late decelerations	t
18	52·0	7·26	Late decelerations	t
19	25·0	7·26	Late decelerations	t
21	37·0	7·19	Late decelerations	
22	100·0	7·26	Late decelerations	
Mean	57·7	7·24		

ECS = Electrocerebral silence
FHR = Fetal heart rate
v = loss of beat-to-beat variability
t = baseline tachycardia

in the fetal EEG in Group A was 1·7, in Group B with FHR decelerations but normal pH values it rose to 11·0 and in Group C with both FHR decelerations and low or falling pH values it rose to 57·7.

DISCUSSION

Rosen *et al* (1973*a*) recorded the fetal EEG before, during and after forceps rotation and traction and found that flattening of the EEG occurred at a traction pressure of 25 mm Hg. This change was not seen during maternal bearing down in normal spontaneous delivery.

Mann *et al* (1972) recorded the fetal EEG in chronic fetal lamb preparations whilst applying varying forces to the fetal head until the fetal EEG became 'flat'. They concluded that head compression, by causing cerebral ischaemia, was responsible for the 'flat' EEG but that the force applied was probably of greater magnitude than that occurring in clinical situations. In several experiments the fetal EEG was unaffected by the force applied and these experiments were not included in the analysis.

The fetuses in our series were monitored during the conduct of labour and those with head compression were identified by retrospective analysis of clinical and radiological features (Chassar Moir, 1971). The patients in our series did not have the fetal EEG monitored when external mechanical pressures were applied to the fetal head. It may be possible that excessive compressive forces or forceps traction and rotation cause ischaemia and ECS in the fetal EEG, but we could not demonstrate any statistical significance in the percentage of ECS occurring in the fetal EEG between a group of essentially normal labours and a group with fetal head compression.

In our series the fetal EEG was analysed in 10 second epochs and correlated with the onset, duration and termination of many hundreds of uterine contractions on a time-sequence basis. If head compression did cause ECS then this change should have been demonstrated in relation to the peak of uterine contractions when the head was being driven down into the lower segment and bony pelvis. It was not possible to demonstrate that periods of ECS were specifically associated with uterine contractions even when oxytocin augmentation was instituted.

Rosen *et al* (1973*b*) described deterioration in the fetal EEG to a silent state in association with late or variable decelerations but not early decelerations. Furthermore, he described a sequence of fetal EEG change progressing in a characteristic manner to a 'flat trace' as the heart rate deceleration developed and returning to normality as the FHR returned to its baseline value.

Our data does not confirm this association of change between these two fetal parameters and demonstrates that early decelerations may also be associated with prolonged silence in the fetal EEG. The appearance of FHR deceleration patterns was significantly related to increasing percentages of ECS in the fetal EEG but both FHR decelerations and mild deterioration in the fetal EEG occurred together and preceded the more slowly changing fall in scalp blood pH values. No consistent relationship could be detected between each deceleration sequence and periods of ECS in the fetal EEG on close scrutiny of simultaneously recorded traces. This finding suggests that FHR decelerations do not cause ECS and vice versa, but rather that changes in these two indices are caused by the acidaemic process which finally leads to a fall in blood pH values. It was noteworthy that late deceleration patterns occurred in six fetuses with normal scalp pH values and only moderately raised percentages of ECS.

Acidosis from whatever cause is the major factor associated with fetal EEG deterioration and may arise secondary to and co-exist with cephalopelvic disproportion amongst the group of labours in which fetal head compression occurred, fetuses with normal scalp blood pH values had minimal EEG change and those with low or falling pH values demonstrated considerable EEG change. Labours which were terminated by abdominal delivery for obstructed labour and in which the fetal scalp blood pH remained within normal limits were not associated with marked change in the fetal EEG.

It would seem that the human fetal head is relatively well adapted to withstand the compressive forces of normal labour and that neurological change occurs in response to fetal acidosis. Purvis *et al* (1975) showed that in the absence of asphyxia the long term prognosis of infants with trauma is good. Our work on the fetal EEG may explain why.

In the human fetus, without the measurement of PO_2, it would appear that acidosis is the major factor associated with the development of a silent fetal EEG. Beard *et al* (1967), Wood *et al* (1967) and Kubli and Berg (1965) suggested that a scalp blood pH of 7·20 units or less identified the asphyxiated fetus. The data presented here shows that even at a scalp blood pH of 7·25 marked fetal EEG change is present. Scalp blood pH without the measurement of PCO_2 and PO_2 is only an indirect measure of hypoxaemia. Intermittent hypoxaemia could cause ECS with comparatively little change in pH.

In summary, the process leading to acidosis during labour in the human fetus was responsible for early deterioration in the fetal EEG to a state of electrocerebral silence. Moderate fetal EEG deterioration coincided with the development of FHR deceleration patterns despite maintenance of normal scalp blood pH values.

The fetal EEG was most severely affected in fetuses with both low or falling scalp blood pH values and FHR deceleration patterns.

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REAL-TIME ULTRASOUND OBSERVATION OF FETAL ACTIVITY IN LABOUR

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Summary

Fetal activity was observed by realtime ultrasound for one hour in 25 patients in labour. Fetal respiratory movement, although reduced compared to the antenatal period, was observed in 13 patients. In three patients, hiccough-like movements were noted. Fetal body and/or extremity movements were observed in all patients (mean 41/hour) and frequently occurred in episodes. These bursts of movements were significantly correlated with contractions and fetal heart rate (FHR) accelerations. There was a significant association between absent or reduced number of fetal respiratory movements and/or hiccoughs (10 or less/hour) and an abnormal FHR. Absence of any type of fetal activity was also significantly associated with an abnormal FHR. The correlation between fetal activity and conventional methods of fetal assessment is discussed.

AT the present time, ultrasound is the optimal method for observation of human fetal activity, including respiratory, body and limb movements. Short- and long-term studies have been performed antenatally, and attempts have been made to correlate the findings with fetal well-being (Bots *et al*, 1976; Fox, 1976; Marsal *et al*, 1976; Patrick *et al*, 1978). However, we were unable to find any reports on the application of ultrasound in labour.

This study was designed to use ultrasound to obtain basic information on the type and frequency of fetal activity in labour. In addition, attempts were made to correlate the findings with conventional means of assessment of fetal

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wellbeing, namely continuous monitoring of fetal heart rate (FHR), fetal acid-base and endocrine studies (total serum oestrogens and human placental lactogen).

PATIENTS AND METHODS

In 25 patients, the fetus was observed by ultrasound, using a linear array, 3·5 MHz transducer (Advanced Diagnostic Research Corporation, Tempe, Arizona), for a period of one hour, between 0800 and 1800 hours. The study was performed during the early part of active labour with the patient in the left lateral position. Patrick *et al* (1978) reported that, with longitudinal monitoring of the fetus, gross fetal movements interfere with the recording of fetal respiratory movements. We therefore decided to observe a transverse section of the fetal abdomen at the level of the umbilical vein. At this level, fetal respiratory movements and hiccoughs are characterized by a change in diameter and tissue pattern of the crossection. Fetal respiratory movements can be observed even when the fetus moves. Readjustment of the transducer is rarely necessary.

Of the 25 patients, 13 were primiparae and 12 multiparae. In nine patients, the onset of labour occurred spontaneously, while 16 were induced by artificial rupture of the membranes and an intravenous oxytocin infusion. The mean gestational age was 39 weeks (range 35 to 42 weeks), and was not significantly different ($p < 0 \cdot 05$) in patients with spontaneous and induced labour.

The complications of pregnancy present in these 25 patients are listed in Table I.

There were only two smokers in the group, and none of the patients had smoked within three hours of the onset of the study. They had fasted for an average of 16 hours, and had rested in bed for at least one hour. Medications were used by 23 of the 25 patients within 24 hours and are listed in Table II.

Fetal respiratory movements, body and extremity movements and hiccoughs were monitored using scale 2 magnification. The image was transmitted by a video-camera through a custom designed mirror system (Photographic Consulting and Engineering Services, Delta, B.C.) to a television monitor for instant analysis and to a videotape recorder for permanent recording.

TABLE I

Complications of pregnancy in 25 patients

Complication	Number of patients
None	6
Hypertension/pre-eclampsia	7
Diabetes (1), gestational diabetes (2)	3
Postmaturity	2
Rhesus isoimmunization	2
Suspected intrauterine growth retardation	2
Miscellaneous (premature labour; premature rupture of membranes; renal disease)	3

TABLE II

Medications used in 25 patients within 24 hours of the study

Medication	Time interval between medication and study onset (hours)	Number of patients*
5 per cent dextrose/water (intravenous)	1†	20
Barbiturates (oral)	12	5
Tranquilizers (oral)	12	2
Ampicillin (intravenous)	3	1
Epidural	1†	2

* 7 patients received more than one medication.
† Used throughout study.

Fetal respiratory movements, body and extremity movements and hiccoughs were recorded with an event marker on one channel of a Beckman RP Dynograph. The second channel was used to record the FHR which was transmitted by telemetry (Graystone and Towell, 1971). In addition, simultaneous recordings of FHR and uterine activity were made with a conventional cardiotocograph, using direct fetal ECG monitoring and intrauterine pressure catheters. Fetal blood sampling for pH and blood gases was done in 12 patients because of an abnormal FHR tracing.

At the onset of the study a maternal venous blood sample was drawn and analyzed for serum glucose, total serum oestrogens (Radiodiagnostic Kit, Diagnostic Biochemicals, London, Ontario) and human placental lactogen

(Radioimmunoassay Kit, Amersham, Oakville, Ontario).

At delivery, samples of umbilical arterial and venous blood were collected and analyzed for pH and blood gases. Apgar scores at one and five minutes, fetal weight and the progress of the infant in the nursery were recorded.

Analysis of fetal respiratory movements included the total number and the total time spent in respiratory activity. Apnoea was defined as the absence of fetal respiratory movements for more than six seconds.

Fetal movement was defined as any fetal body and/or extremity movement identified. Fetal movement bursts were defined as groups of movements with a frequency of less than 0·1 Hertz.

Sudden spasmodic fetal movements, occurring at regular intervals of two to ten seconds over several minutes, simulating large amplitude respiratory movements were termed hiccoughs, and the number and duration of an episode of these movements were recorded.

Total time without fetal activity was defined as the time in minutes in which the fetus was completely quiescent.

The FHR recordings were subsequently analysed and accelerations defined as an increase of the fetal heart rate of at least ten beats/minute above the baseline.

For statistical analysis a Chi square test with the Yates correction and the correlation coefficient were used.

RESULTS

Labour and delivery data are recorded in Table III.

Fetal respiratory movements were observed in 13 out of 25 patients (56 per cent). They were either isolated or occurred in episodes lasting up to 30 seconds. The average duration of fetal respiratory movements was 60 seconds (range 6 to 191 seconds) and the average number of fetal respiratory movements per hour was 48 (range 2 to 167). Fetal respiratory movements were occasionally observed in association with other fetal movements and hiccoughs.

Episodes of hiccoughs were noted in three fetuses. They lasted 245, 350 and 900 seconds with the total number of hiccoughs ranging from 68 to 254, and were associated with sharp spikes on the FHR tracing.

Body and extremity movements were identified in all fetuses; an average of 41 movements (range 10 to 94 movements) was observed in the hour of recording. This represents a minimum figure, as it is possible that some minor extremity or head movements may have been missed. The movements frequently occurred in episodes with

TABLE III

Labour and delivery data (25 patients)

	Mean	Range
Cervical dilatation (cm)	3·8	2–9
Study-delivery interval (hrs)	3·6	1–9
Fetal blood pH (n = 12)	7·36	7·27–7·40
Serum glucose* (mmol/l) (n = 22)	4·98	4·29–7·04
Serum oestrogens (nmol/l)	315·4	122·5–770·0
HPL (nmol/l)	330·2	134·9–637·1
Birth weight (kg)†	3·45	2·13–4·67
Umbilical artery pH‡	7·30	7·02–7·39
Apgar score§ 1 minute	7·7	0–10
5 minutes	8·8	4–10

* 3 diabetic patients excluded.

† One infant fell below the 10th centile (Lubchenco *et al*, 1963).

‡ pH less than 7·25 in 1 patient (FBS pH 7·32; UA pH 7·02; Apgar score 8/9; Nuchal cord).

§ Apgar score 7 or less at (i) 1 minute (n = 5); (ii) at 5 minutes (n = 4).

TABLE IV

Relation between number of fetal respiratory movements (FRM) and/or hiccoughs and FHR (25 patients)

	No. of patients with FRM and/or hiccoughs		
FHR recording	0 to 10/hour	more than 10/hour	
During study hour			
Normal	3	11	
Abnormal*	10	1	
			$\chi^2 = 9{\cdot}3$; $p < 0{\cdot}01$;
Throughout labour			
Normal	5	11	
Abnormal*	8	1	
			$\chi^2 = 5{\cdot}5$; $p < 0{\cdot}02$

* Poor variability; severe variable or late decelerations; bradycardia.

TABLE V

Relation between total time without fetal activity and FHR throughout labour (25 patients)

	No. of patients without fetal activity		
FHR recording throughout labour	45 minutes or less	more than 45 minutes	
Normal	15	1	
Abnormal*	3	6	
			$\chi^2 = 7{\cdot}7$; $p < 0{\cdot}01$;

* Poor variability; severe variable or late decelerations; bradycardia.

an average of 20 bursts per hour (range 7 to 39 bursts per hour). The correlation between bursts of activity and FHR accelerations ($r = +0{\cdot}9548$) was highly significant ($p < 0{\cdot}001$).

A reduced incidence of fetal respiratory movements and/or hiccoughs was significantly associated with an abnormal FHR recording during the study hour or at any time during the course of labour (Table IV). Absence of fetal activity for a total of more than 45 of the 60 minutes of observation was also significantly associated with an abnormal FHR tracing (Table V).

DISCUSSION

Various types of fetal activity can be observed throughout labour. The incidence of fetal respiratory movements and of fetal activity in general is considerably reduced in labour in comparison to the antenatal period (unpublished observations). In our experience, some activity is maintained and its degree may reflect fetal well-being as assessed by the FHR recording.

No correlation was found between the number of fetal respiratory movements and the fetal outcome as judged by Apgar score at one and five minutes or the pH of the umbilical arterial blood. However, it is of interest to note that in two cases, one with severe acidosis and the other with severe depression, fetal respiratory movements were decreased or absent. Fetal activity and outcome, on the other hand, showed no relation to intrapartum levels of maternal total serum oestrogen or placental lactogen.

Fetal movements were observed in all patients. The significant correlation with contractions may indicate that contractions stimulate the fetus to move. Fetal movements also correlate with an increase of FHR. On several occasions, it was noted that even during variable decelerations, fetal movements appeared to be associated with an increase of the FHR. This may indicate, that an increase in FHR is triggered by fetal movement itself or that both events are related to a common central mechanism.

Absence of any activity for more than 45 of the 60 minutes of observation was significantly correlated with an abnormal FHR throughout the course of labour. Here again, fetal activity may reflect fetal well-being.

Although the number of patients is small, it appears that the healthy fetus during labour has short episodes of respiratory movements and reacts to contractions with bursts of movements, associated with an increase in the FHR. Reduced or absent fetal respiratory movements and decreased total activity may indicate a fetus at risk. Further work is required to confirm these results.

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PROSTAGLANDINS IN UMBILICAL PLASMA AT ELECTIVE CAESAREAN SECTION

BY

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Summary

Prostaglandin E (PGE), prostaglandin F (PGF) and 13,14-dihydro-15-keto-prostaglandin F (PGFM) have been measured in umbilical cord plasma obtained immediately after delivery by elective Caesarean section. Umbilical venous plasma levels of PGE were significantly greater than the corresponding arterial levels ($p < 0{\cdot}02$); there were no significant arterio-venous differences for PGF or PGFM. In general, concentrations of PGFM exceeded those of PGE which in turn were greater than concentrations of PGF. Umbilical venous levels of PGE and both arterial and venous levels of PGF and PGFM were significantly greater after spontaneous labour with vaginal delivery than after elective Caesarean section.

THE concentrations of PGE and PGF have been reported to be greater in umbilical plasma and serum than in maternal plasma and serum (Hertelendy *et al*, 1973; Craft *et al*, 1973; Challis *et al*, 1974; Pokoly and Jordan, 1975). We have recently described similar findings (Mitchell *et al*, 1978*a*) although our measured levels were lower than previous investigations and we could also demonstrate a significant arterio-venous difference for PGE with raised venous levels. It was suggested that the placenta may be a significant source of PGE in the fetal circulation, as it is in sheep (Mitchell and Flint, 1978). PGE has been implicated in the control of fetal and placental haemodynamics (Novy *et al*, 1974; Rankin and Phernetton, 1976*a* and *b*) and the maintenance of the tone of the ductus arteriosus (Coceani *et al*, 1976). Hence tonic secretion of PGE by the placenta may be of crucial importance for fetal wellbeing. Since the concentration of PGE in the circulation of the fetal sheep rises shortly before delivery (Challis *et al*, 1976), it is possible that the arterio-venous difference for PGE in umbilical plasma previously demonstrated in human pregnancy after the spontaneous onset of labour and vaginal delivery (Mitchell *et al*, 1978*a*) was associated more with labour itself rather than being a feature of the fetal circulation in late pregnancy. In order to test the validity of this, we measured the concentrations of prostaglandins in umbilical arterial and venous plasma following delivery at term by elective Caesarean section and compared these measurements with those previously described in umbilical plasma after vaginal delivery.

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Patients and Methods

Full and informed consent was obtained from the 10 women in this study. All were delivered of liveborn infants by elective Caesarean section under general anaesthesia at term (range, 38 to 41 weeks). Caesarean section was performed because of cephalopelvic disproportion, presentation by the breech or a previous Caesarean section. Baby and placental weights (g) were (mean±SEM, n = 10) 3328±126 and 525±22 respectively. At delivery, blood samples were obtained from the umbilical artery and vein before clamping, whilst pharangeal aspiration of the infant was performed. Particular care was taken to avoid manipulation or trauma to the cord.

Blood was taken into ice cold tubes containing acetylsalicylic acid and EDTA and centrifuged immediately at 1500 *g* and 10°C. Plasma was stored at −20°C until analysis. PGE, PGF and PGFM were measured by radioimmunoassays which have been fully described and validated previously (Mitchell *et al*, 1978*a*; Mitchell *et al*, 1978*b*).

Statistical analyses were performed using the Mann Whitney Rank Sum Test (for unpaired samples) and the Wilcoxon Signed Rank Test (for paired samples).

Results

The concentrations of prostaglandins in umbilical arterial and venous plasma taken at elective Caesarean section are shown in Figure 1. There was a significant arterio-venous difference for PGE ($p < 0{\cdot}02$) but not for PGF ($p > 0{\cdot}1$) or PGFM ($p > 0{\cdot}1$). The concentration of PGE exceeded that of PGF in venous ($p < 0{\cdot}01$) but not arterial ($p > 0{\cdot}1$) plasma. PGFM levels were consistently greater than PGF levels in both arterial ($p < 0{\cdot}01$) and venous ($p < 0{\cdot}01$) plasma and were also greater than levels of PGE in arterial plasma ($p < 0{\cdot}01$).

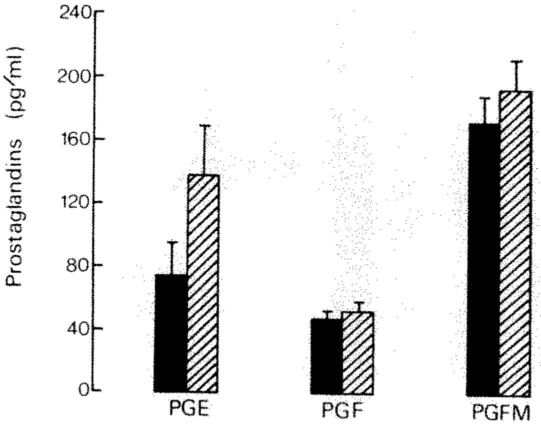

Fig. 1

The concentrations of prostaglandins (mean±SEM) in umbilical arterial (filled bars) and venous (hatched bars) plasma taken from ten patients immediately after delivery by elective Caesarean section.

Table I

Concentrations of prostaglandins in umbilical plasma after delivery at term

Group and source of prostaglandin		Concentration of prostaglandin (pg/ml) Mean±SEM	
		Elective Caesarean section (n = 10)	Spontaneous labour and vaginal delivery (n = 12)†
PGE	Artery	74±20	109± 27
	Vein	138±31	242± 25*
PGF	Artery	47± 5	80± 10*
	Vein	52± 6	88± 11**
PGFM	Artery	172±16	640±180**
	Vein	193±18	631±107**

Statistical difference (by Mann Whitney Rank Sum Test) compared to elective Caesarean section.

* $p < 0{\cdot}02$; ** $p < 0{\cdot}01$

† From Mitchell *et al* (1978*a*).

The concentration of PGE in umbilical venous plasma after vaginal delivery previously reported is greater than at elective Caesarean section ($p < 0{\cdot}02$; Table I) although there was no difference between arterial plasma concentrations ($p > 0{\cdot}1$). Concentrations of

PGF and PGFM in both arterial and venous plasma were significantly greater after spontaneous vaginal delivery.

DISCUSSION

These results demonstrate that at term before the onset of labour there is a significant arterio-venous difference for PGE across the umbilical circulation, venous levels being higher. Hence the similar finding after spontaneous labour and vaginal delivery (Mitchell *et al*, 1978*a*) was not entirely a labour-associated phenomenon. It should be noted, however, that arterial concentrations of PGE were similar whether delivery occurred vaginally or by elective Caesarean section whereas venous concentrations were significantly raised after vaginal delivery (Table I). This suggests that labour is associated with an enhancement of the magnitude of the arterio-venous difference, due most probably to increased placental production of prostaglandins, although there are other possible reasons. For example, the events preceding elective Caesarean section such as bed rest, starvation, premedication drugs and general anaesthesia could have effects on prostaglandin concentrations and are currently being investigated.

Since concentrations of both PGF and PGFM were also raised after spontaneous vaginal delivery, it is likely that the raised venous levels of PGE reflect an increase in its rate of synthesis rather than a reduction in its rate of catabolism. Certainly a previous study could not demonstrate any change with labour in the activities of various intrauterine tissues with regard to prostaglandin catabolism (Keirse *et al*, 1976).

The lack of a significant arterio-venous difference for PGF is puzzling but it is possible that the fetus has, and develops, a greater number of receptors for PGE or better systems for its selective uptake and/or metabolism. PGE has been shown to be a far better substrate for 15-hydroxyprostaglandin dehydrogenase than PGF (Keirse and Turnbull, 1975).

The arterio-venous difference for PGE before the onset of labour may suggest that during late pregnancy PGE of placental origin has specific functions as previously suggested from experiments on animals (Novy *et al*, 1974; Rankin and Phernetton, 1976*a* and *b*). Whether this arterio-venous difference for PGE is a feature of late pregnancy or occurs throughout gestation remains to be established.

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SEQUENTIAL CHANGES IN THE HUMAN RENIN-ANGIOTENSIN SYSTEM FOLLOWING THERAPEUTIC TERMINATION OF PREGNANCY

BY

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Summary

Plasma renin and angiotensin II levels were measured in nine patients immediately before and at half-hourly intervals in the four hours following therapeutic termination of pregnancy. There was a small fall in renin and angiotensin II levels over the first 1 to 2 hours, followed by a slight increase. The magnitude of these effects was much smaller than those previously seen following normal delivery. It is concluded that in early pregnancy maternal, rather than feto-placental, factors are controlling the renin-angiotensin system.

Plasma renin activity has been reported to be significantly increased in the luteal phase of the menstrual cycle (Brown *et al*, 1964*a*; Skinner *et al*, 1969; Robertson *et al*, 1971; Sundsfjord and Aakvaag, 1973; Katz *et al*, 1975). Angiotensin II levels are also increased at this time (Sundsfjord and Aakvaag, 1970; Robertson *et al*, 1971). Should conception occur, these elevated levels of renin and angiotensin II are maintained (Sundsfjord and Aakvaag, 1973; Weir *et al*, 1976). High levels of plasma renin activity, total renin concentration and angiotensin II have been reported in the first trimester of pregnancy (Brown *et al*, 1964*b*; Skinner *et al*, 1972; Weir *et al*, 1976; Lammintausta and Erkkola, 1977) but the stimulus for and function of these raised levels remain controversial. In a recent paper (Broughton Pipkin *et al*, 1978) we reported that the high levels of plasma renin activity and concentration and angiotensin II seen at delivery fell rapidly over the first two hours post partum to levels within the non-pregnant range. This initial fall was followed by a rise in all three parameters and it was suggested that these changes were consequent upon removal from the maternal circulation of renin, and possibly angiotensin, of feto-placental origin. The later increase was tentatively ascribed to an overshoot of renal renin production following the release from feed-back suppression evoked by such 'pregnancy renin'.

The present study was designed to establish whether similar hormonal changes could be evoked by termination of pregnancy in the first trimester, at a time when the renin-angiotensin system is known to be active.

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PATIENTS AND METHODS

Nine patients were studied immediately before, and for the first four hours after, therapeutic termination of pregnancy. Seven patients were primigravidae; the remaining two were gravida 3 and 4 respectively. Basic clinical details are summarized in Table I. One patient gave a family history of hypertension, the remainder gave no positive personal or family history of hypertension or renal or metabolic disease.

All patients received an intravenous infusion of 5 per cent dextrose at a rate of 30 drops/minute throughout the study via an indwelling teflon cannula to which was attached a three-way tap. Blood pressure measurements were made using an Arteriosonde blood pressure recorder (Roche Medical Electronics).

Blood pressure was recorded and a blood sample was obtained from an antecubital vein after the patient had rested supine for a minimum of 20 minutes. Pregnancies were terminated by suction evacuation of the uterine contents using a Clements pump following paracervical block using 15 to 20 ml of 1 per cent Xylocaine without adrenalin. Blood pressure measurements and blood samples were taken within 3 to 14 minutes of the termination of pregnancy and at 30-minute intervals thereafter for the following four hours. Plasma renin activity and concentration and plasma angiotensin II levels were measured as previously described (Broughton Pipkin *et al*, 1978).

All values are quotes as Mean ± SEM throughout. Student's 't' test or a paired 't' test has been used as appropriate to test for the significance of the difference between two means. Linear regression coefficients have been calculated by the method of least squares.

TABLE I

Clinical details of patients in our study

Name	Age (years)	Gravida	Gestational age (weeks)	Initial blood pressure (mm/Hg)
A.L.	16	1	8	118/70
A.H.	18	1	9	110/58
G.C.	19	1	11	118/80
S.G.	19	1	~9*	125/65
L.C.	20	1	9	105/60
K.B.	21	1	10	130/70
M.D.	24	1	9	110/74
P.R.	34	3	10	120/72
P.L.	35	4	9	125/70

* 8 months amenorrhoea after stopping oral contraception.

RESULTS

Mean plasma renin activity and concentration and angiotensin II levels, before and four hours after termination of pregnancy, are summarized in Table II. There was a small fall in plasma renin concentration and angiotensin II levels over the first hour following termination to levels 30 to 35 per cent lower than those initially seen; this difference was not statistically significant (Fig. 1). Plasma renin activity fell by a similar amount over the two hours following termination, but this fall was also not significant (Fig. 1).

Following these initial small falls, plasma renin concentration, activity and angiotensin II levels rose slightly to similar or slightly higher levels than those initially observed. Although the proportional fall in hormone level with time in the first period, and rise in the second, failed to achieve statistical significance, the change in slope from the first to the second period was significant for both plasma renin activity and plasma renin concentration (P <0·05 for both).

Plasma renin activity and concentration were highly significantly related overall (r = 0·7381; P <0·001). There was also a high degree of correlation between plasma renin activity and angiotensin II (r = 0·3397; P <0·005) and plasma renin concentration and angiotensin II (r = 0·3603; P <0·002).

TABLE II

Plasma renin activity and concentration and angiotensin II levels before and four hours after termination of pregnancy

	Plasma renin activity (ng/ml/hour) Mean ± SEM	Plasma renin concentration (ng/ml/hour) Mean ± SEM	Plasma angiotensin II (pg/ml) Mean ± SEM
Before termination	1·73 ± 0·37	3·49 ± 0·45	56·7 ± 16·3
4 hours after termination	1·99 ± 0·40	3·45 ± 0·65	78·5 ± 15·0

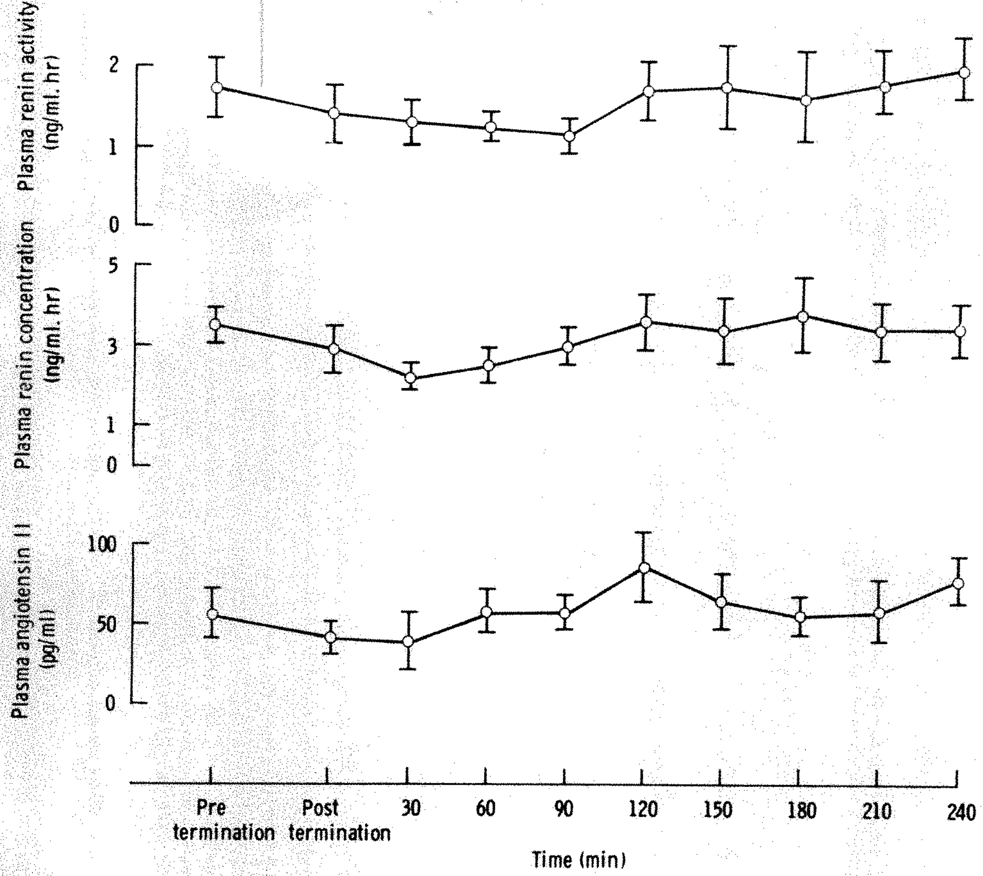

FIG. 1

The observed changes in plasma renin activity and concentration and angiotensin II in the four hours following the termination of pregnancy; these were small.

Overall there was a highly significant inverse relation between diastolic blood pressure and simultaneously measured angiotensin II levels (r = −0·493; P <0·001). Similar, albeit less significant, relationships were found between both plasma renin activity and plasma renin concentration and blood pressure (r = −0·3047, P <0·005; r = −0·2707, P <0·02 respectively). Partial correlation coefficients were calculated for the relation between the two renin measurements and diastolic blood pressure when the effect of their inter-relation with angiotensin II was held constant. Under these circumstances the relation between renin levels and diastolic blood pressure was no longer statistically significant. Conversely, correcting for the observed relation between the two renin measurements and angiotensin II did not materially affect the correlation between diastolic blood pressure and angiotensin II levels (r = −0·4300; P <0·001).

DISCUSSION

The stimulus for the increased activity of the renin-angiotensin system in the luteal phase of the menstrual cycle and for its hyperactivity from the very early stages of pregnancy is still a matter for controversy. Sundsfjord and Aakvaag (1970) have suggested that the peri-ovulatory surge in progesterone may, by its mild natriuretic effect, stimulate the renin-angiotensin system. Certainly, there is a marked increase in urinary sodium excretion following ovulation (Weir *et al*, 1976) which would be expected to stimulate the renin-angiotensin system and, other things being equal, aldosterone production. A direct correlation has not yet been demonstrated and the available data are scanty.

An alternative hypothesis invokes an increase in renin substrate as a consequence of increased oestrogen secretion stimulating hepatic protein synthesis. This seems somewhat less likely since changes in plasma oestradiol levels are temporally coincident with the observed changes in renin levels, instead of antedating them, as might be expected. Furthermore, the elegant kinetic studies of Skinner *et al* (1969) showed the rise in plasma renin activity during the luteal phase to be due to an increase in the concentration of the enzyme itself, rather than to an increase in its substrate.

In early pregnancy ovarian progesterone production is probably vital, and the placenta appears not to exert a major synthesizing role until after at least 56 days gestation (Yoshimi *et al*, 1969). Similarly, pregnancy oestrogens appear to be synthesized by the feto-placental unit, and their production at the time of this study (8 to 11 weeks gestation) is extremely low. Thus the two factors postulated as stimuli for increased renin production are still largely under maternal control rather than feto-placental control at the period of gestation at which this study was done.

In the two hours immediately following normal delivery there is a marked fall of renin activity and concentration and angiotensin II concentration to levels within the non-pregnant range. This fall is followed by an increase in all three parameters which persists for at least the next two hours (Broughton Pipkin *et al*, 1978). The time course of the changes in plasma renin level is consistent with the known half-life of renin in the circulation, and it has been postulated that the fall represents a clearance from the circulation of renin of feto-placental origin. It was further postulated that this 'pregnancy renin' might be capable of the suppression of renal renin secretion and that the subsequent rebound in renin levels represented an escape from such suppression with a temporary overshoot in production.

The data presented in this paper suggest that any feto-placental contribution to maternal levels of renin and angiotensin must be small in the first trimester of pregnancy. Brown *et al* (1964*b*) found an extremely high total renin level in amniotic fluid obtained at eleven weeks of gestation. However, total plasma renin was measured in eight of the patients described in this paper, and had an initial value of $40 \cdot 7 \pm 5 \cdot 0$ ng/ml (mean ± SEM), not significantly different from that measured at delivery ($44 \cdot 9 \pm 9 \cdot 1$ ng/ml). Molteni *et al* (1974) found that although fetal renal renin could be demonstrated from 58 days gestation, the levels found were lowest in the youngest fetus and highest in those of 104 to 108 days gestation. Comparable data are not, to our knowledge, available for normal term fetus.

The hypothesis, that at this stage of pregnancy maternal, rather than feto-placental factors, are controlling the renin-angiotensin system, is lent further indirect support by the observed inverse relation between diastolic blood pressure and simultaneously measured angiotensin II levels. Such a relation implies that at least one of the normal stimuli for renin release in the non-pregnant state, namely low renal perfusion pressure resulting from a low systemic blood pressure, is functioning in early pregnancy. Indeed, one patient who suffered a transient hypotensive episode (diastolic blood pressure 42 mm Hg) had, a few minutes later, a plasma AII concentration of 195 pg/ml.

This inverse relation is in marked contrast to that found in late pregnancy where a strongly positive relation has been demonstrated, both in man (Broughton Pipkin and Symonds, 1977) and in sheep (Broughton Pipkin and O'Brien, 1978). The effect of a specific angiotensin antagonist in pregnant sheep suggests that in this species at least the relationship may be causal (Broughton Pipkin and O'Brien, 1978).

Thus various facets of the control of the renin-angiotensin system appear to differ markedly in early and late pregnancy. The data presented in this paper show only a pale shadow of the changes observed following the normal termination of pregnancy. They suggest that at this stage the maternal kidney may still be the major source of circulating renin, subject to the usual stimuli of changing sodium excretion and changing blood pressure. The period in which the apparent changeover to a more autonomous production of feto-placental 'pregnancy renin' takes place cannot yet be defined. Indeed, unless it becomes possible to differentiate between renal and 'pregnancy' renin, it may be impossible to define.

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ULTRASOUND EVIDENCE OF EARLY PREGNANCY FAILURE IN PATIENTS WITH MULTIPLE CONCEPTIONS

BY

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Summary

I report a review of 1500 pregnant patients: 30 of them (or 1 in 50) were thought to have multiple pregnancies when examined by ultrasound in the first trimester. Only 14 of the 30 patients (46·7 per cent) produced live infants: 13 twins and one set of triplets. Twelve (40 per cent) had bleeding in early pregnancy and 11 of these had a spontaneous abortion. Five of seven patients found to have a normal pregnancy and a coexistent blighted ovum, ultimately delivered a single live infant.

THE use of diagnostic ultrasound has in recent years proved to be helpful in the evaluation of early pregnancy failure (Donald *et al*, 1972; Duff, 1975; Robinson, 1975*a* and *b*). There is, however, little information about the incidence and outcome of multiple pregnancy diagnosed by ultrasound scanning in the first trimester. Hellman *et al* (1973), in their series of 140 at-risk early pregnancies, found 22 patients (15·7 per cent) with twin gestation sacs of whom 14 (63·7 per cent) aborted. Robinson (1975*a*) suggested that twin conceptions were more common than was generally appreciated and that many of the gestation sacs contained blighted ova, while Levi (1976) reported that only 29 per cent of a series of patients with twin pregnancies diagnosed before ten weeks eventually gave birth to two live children. More recently, Robinson and Caines (1977) reported that only 14 out of 30 patients (47 per cent) with twin gestation sacs diagnosed in the first trimester delivered two live children. The purpose of this study was to investigate the incidence and outcome of 30 patients with multiple gestation sacs diagnosed by ultrasound scanning in the first trimester of pregnancy in a hospital practice.

METHODS

All ultrasound examinations were performed with Diasonograph NE 4102 (Nuclear Enterprises Limited, Edinburgh). Ultrasound diagnosis of a normal viable multiple pregnancy in the first trimester was based on the identification of fetal heart movements within each distinct group of fetal echoes (Robinson, 1972). The ultrasound criteria used for the diagnosis of failure of pregnancy were as described by Robinson (1975*b*). Ultrasound scanning was repeated at intervals of two weeks until the outcome of the multiple gestation sacs was known.

RESULTS

During a period of two years between 1976 and 1977, 1500 patients were examined by ultrasound in the first trimester of pregnancy; 30 of them (or 1 in 50) were found to have multiple gestation sacs (29 twins and one set of triplets).

Table I shows the incidence of complications associated with multiple gestation sacs diagnosed by ultrasound scanning in the first trimester of pregnancy and its outcome.

TABLE I

Outcome of multiple gestation sacs diagnosed by ultrasound examination in the first trimester

Outcome	Number	Incidence of bleeding	Incidence of abortion	Incidence of continuing pregnancy	
				Singleton	Multiple
Blighted ova	5	5	5	0	0
Missed abortion	3	3	3	0	0
Blighted ova and co-existent normal pregnancy	7	2	2	5	0
Multiple pregnancy	15	2	1	0	14
Total	30	12 (40%)	11 (36·7%)	5 (16·7%)	14 (46·7%)

Blighted ova

Absence of the fetal echo and fetal heart movements were observed in 5 of 30 patients (17 per cent) with an early diagnosis of multiple sacs at eight to ten weeks gestation. All five were admitted with bleeding and went on to abort spontaneously between 11 and 13 weeks of gestation.

Missed abortion

Three of 30 patients (10 per cent) had twin gestation sacs with fetal echo and fetal heart movement compatible with nine weeks gestation at first scan, but were later admitted with bleeding between 10 and 11 weeks gestation. Repeat scans on admission and a week later in these three showed no clear fetal echo or heart movement in either sac. All three patients had a spontaneous abortion following this admission.

Blighted ovum and normal pregnancy

Seven of 30 patients (23·3 per cent) had normal twin sacs at 6 weeks gestation. Repeated scans between 8 and 12 weeks gestation showed that while one of the twin sacs was developing normally with fetal echos and fetal heart movements appropriate to the period of gestation, the second sac was smaller and showed no fetal echos or heart movements. Two of these seven patients were admitted with bleeding at 10 weeks and eventually had a spontaneous abortion at 13 weeks. The remaining five delivered a live single child between 38 and 41 weeks gestation.

Multiple sacs with normally developing fetuses

Fourteen of the remaining 15 patients delivered live infants, 13 sets of twins and one set of triplets, between 35 and 39 weeks gestation. One patient had a spontaneous miscarriage at 24 weeks in spite of attempts to stop uterine contractions.

DISCUSSION

Ultrasound examination provides the unique facility of examining an early pregnancy and can be repeated without risk or inconvenience to the mother and without risk to the fetus. The accurate incidence of twin pregnancies and their final outcome is not known, but it is possible that they are more common than is generally appreciated. A substantial number may be missed, as the abnormal sacs often appear only transiently during the first and early second trimester and the patient may not be referred for ultrasound examinations at this time.

Review of a series of patients studied by ultrasound scans showed that on several occasions, repeated scans late in the second trimester of pregnancy could only demonstrate a single fetus when multiple gestation sacs had been clearly seen earlier in the pregnancy. A longitudinal study of multiple pregnancies was therefore undertaken from the first trimester until the outcome of pregnancy was known. In our unit during a period of two years, 27 sets of twins and one set of triplets were delivered. The diagnosis of multiple pregnancy was made by ultrasound examination during the first trimester on 14 patients who formed the basis for this study, whereas the remaining 14 patients had the diagnosis confirmed in the latter half of the second trimester and were not included.

This study suggests that multiple conceptions in the human are more common than usually reported. It also shows that they are associated with an increased incidence of bleeding in early pregnancy (40 per cent) and a high incidence of abortion (36·7 per cent). Hellman *et al* (1973) reported that 14 of 22 patients (63·7 per cent) with twin gestation sacs aborted and only five (23 per cent) delivered twin infants, whereas Levi (1976) stated that only eight of 28 (28·6 per cent) patients who had the ultrasound diagnosis of twin sacs before 10 weeks gestation, eventually delivered twin infants. Recently Robinson and Caines (1977) reported that 14 of 30 patients (46·7 per cent) with twin gestation sacs diagnosed in the first trimester eventually delivered twin infants and six (20 per cent) aborted spontaneously.

This study reports an incidence of multiple pregnancy of 1 in 50, considerably higher than the commonly accepted figure of 1 in 80. The incidence of bleeding and abortion were high and the presence of blighted ovum did not usually affect the development of a co-existing normal pregnancy adversely. Five of seven patients with a blighted ovum and normally continuing pregnancy were delivered of a single infant between 38 and 41 weeks gestation. Only 14 of 30 patients (46·7 per cent) with a diagnosis of multiple gestation sacs in the first trimester had successful live births.

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PRENATAL AND POSTNATAL MATERNAL LEUCOCYTE ASCORBIC ACID CONCENTRATIONS

BY

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Summary

Leucocyte ascorbic acid was measured in 79 pregnant women in the 34th to 38th week of gestation and on the first, and third or fifth days post partum. No differences were found for time of year or age of the subjects. There was a significantly lower vitamin C status in social classes IV and V. The leucocyte ascorbic acid concentration fell significantly on the first day post partum and rose again to initial values by the fifth day. In late pregnancy 40 per cent of all subjects had leucocyte ascorbic acid concentrations less than the lower limit of normal (100 nmol/10^8 leucocytes). This rose to 75 per cent on the first day post partum.

ALTHOUGH all animals use ascorbic acid as part of their metabolism, only a few species, including primates and guinea pigs, require an exogenous supply of this vitamin to maintain these bodily processes (Chatterjee, 1973) because of a gene causing hypoascorbaemia (Stone, 1966). Even within one species there is a considerable biological variation in ascorbic acid requirement (Yew, 1975). Williams and Deason (1967) found a clinical difference in requirement in a group of guinea pigs and Kline and Eheart (1944) found a four-fold variation in nine women. During pregnancy the requirement for ascorbic acid is increased. When the ovum is released large quantities of ascorbic acid are utilized (Loh and Wilson, 1971) and decreased tissue levels have been reported after fertilization (Wilson and Loh, 1973). There are few reports of deleterious effects of such depressed levels of ascorbic acid in humans, although associations have been reported between decreased ascorbic acid levels and increased neonatal mortality (Toverud, 1936), stillbirth and premature births (Elmby and Becker-Christensen, 1938), decreased birth weight (Wideman *et al*, 1964), and pre-eclampsia (Clemetson and Anderson, 1964), whilst other reports have suggested either no association or only a contributory role for ascorbic acid in these circumstances (Martin *et al*, 1957; Rivers and Devine, 1972). In animal studies, Rivers and Devine (1975) found improved reproductive performance in three generations of guinea pigs fed increased concentrations of ascorbic acid in their diet. The present study examines the maternal concentration of ascorbic acid in leucocytes in the perinatal period and attempts to correlate this with age, social class and birth complications.

PATIENTS AND METHODS

The patients presented at Robroyston Hospital antenatal clinic. All gave informed consent. Initial blood samples were obtained at the last visit to the antenatal clinic between 34 to 38 weeks of pregnancy. Subsequent samples were withdrawn as far as possible on the first day post partum and on the third or fifth day post partum. Samples were withdrawn between 1000 and 1200 hours to minimize the effects of diurnal variation in leucocyte ascorbic acid. Sampling was carried out on two different cohorts of women; and at two times of the year, November to March and July to October, to determine if any seasonal difference existed. As well as leucocyte ascorbic acid measurement, red blood cell count, white blood cell count, packed cell volume and haemoglobin concentration were determined.

All subjects who showed abnormal haematological parameters or who were transfused following delivery were excluded from the study.

Leucocyte ascorbic acid was determined on the buffy coat from blood by the method of Denson and Bowers (1961). The buffy coat was separated on Dextran 125, the ascorbic acid in the cells was extracted into trichloroacetic acid and quantitated by formation of the dinitrophenylhydrazone. Results were expressed as nmol of ascorbic acid/10^8 leucocytes.

Results were analysed statistically by the paired 't' test or by 't' test and by the Mann-Whitney U test and by χ^2 analysis.

RESULTS

In the two periods of the study 79 women in total were examined, 40 in the winter period and 39 in the summer period. No difference could be found in either the age, social class or initial leucocyte ascorbic acid distributions between these two periods following non-parametric

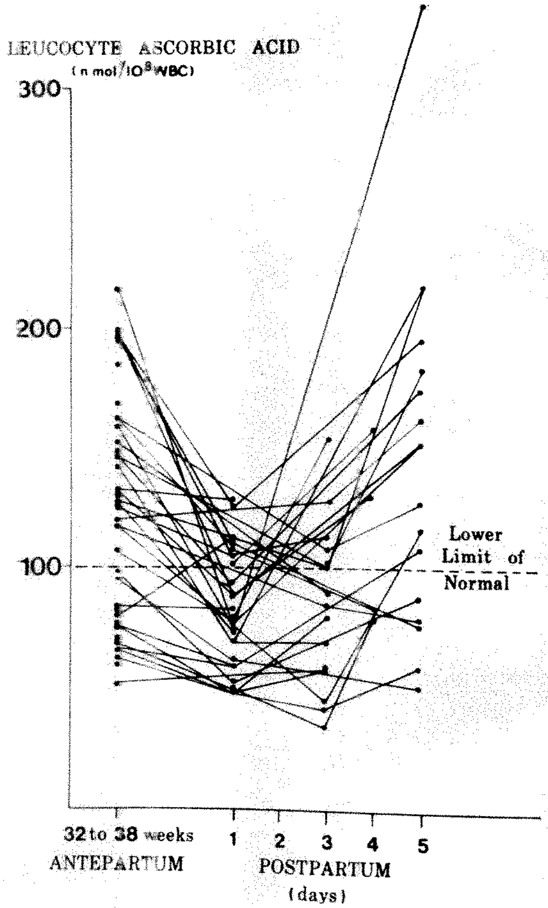

FIG. 1

The changes in leucocyte ascorbic acid concentrations in mothers at delivery. Values for subjects in whom sequential measurements were taken are linked by lines.

TABLE I

Distribution of leucocyte ascorbic acid concentrations in late pregnancy with social class

Social class	I	II	III	IV	V
Number of subjects	2	17	30	18	12
Per cent of subjects below* lower limit of normal (100 nmol/10^8 leucocytes)	10·5		33·3	55·5	75·0
Leucocyte ascorbic acid concentrations (nmol/10^8 leucocytes) Mean ± SD	152·2 ± 38·3		118·4 ± 34·1	103·4 ± 37·1	

* $\chi^2 = 30{\cdot}1$ $p < 0{\cdot}001$ when compared with overall distribution of leucocyte ascorbic aicd values.

analysis and in consequence these are combined for all other analyses. The leucocyte ascorbic acid concentrations showed a consistent depression from the prenatal value to the first measurement post partum with subsequent rises in concentrations up to day 5 (Fig. 1). After birth it was noted that there was a consistent, but not invariable, rise in the white blood cell count with a percentage rise (mean ± SD) of 35·3±44·9. On analysis, however, no convincing relation could be found between the percentage rise in white cell count and the percentage depression in leucocyte ascorbic acid ($r = 0·23$ for 51 pairs of samples). In no patient was the leucocyte count outside the normal range. The social class distribution and the distribution of prenatal leucocyte ascorbic acid values within these social class is shown in Table I. There were insufficient subjects in classes I and II and in IV and V for individual analysis. These pairs were therefore combined and showed a consistent and significant ($\chi^2 = 30·1$, $p < 0·001$) depression in leucocyte ascorbic acid values from the highest to lowest social classes. The lower limit of normal for leucocyte ascorbic acid is 100 nmol/10^8 leucocytes (Denson and Bowers, 1961). Of the 79 women examined, there were 32 (40 per cent) below this limit at initial examination in late pregnancy. One day post partum, of 51 women examined, 39 (76 per cent) were below this limit. Only 51 of the subjects had measurements of leucocyte ascorbic acid taken both initially and on the first day post partum. For these subjects the initial leucocyte ascorbic acid concentration (mean ± SD) was 125·9±44·3 nmol/10^8 leucocytes which fell to a mean of 83·7±22·9 nmol/10^8 leucocytes one day post partum. This was highly significant ($t = 9·058$; $p < 0·001$). The figures for each of the days of the study are shown in Table II. No association could be found between this change in leucocyte ascorbic acid and the change in leucocyte count. Nor was there any association with age either nonparametrically in number of subjects below 100 nmol/10^8 leucocytes or in mean values of leucocyte ascorbic acid (Table III).

Haematological parameters showed no consistent change in those subjects in whom serial measurements were made. In no subject did the haemoglobin value fall below 10·5 g/dl. Values found for the periods are shown in Table IV. No association could be found between leucocyte counts and leucocyte ascorbic acid values

TABLE II

The mean concentration of leucocyte ascorbic acid for each of the days of the study

Day of study	Number of subjects	Leucocyte ascorbic acid (nmol/10^8 leucocytes) Mean ± SD
Initial (prenatal)	79	120·2±40·2
First day post partum	51	83·7±22·9
Third day post partum	40	112·4±38·0
Fourth and fifth day post partum	39	131·6±55·7

TABLE III

Age and seasonal variations in leucocyte ascorbic acid concentrations in late pregnancy

Age (years)		15–20	21–25	26–30	30+
Number of subjects		13	29	20	17
Per cent of subjects below 100 nmol/10^8 leucocytes		46·2	31	50	35·3
Leucocyte ascorbic acid concentrations (nmol/10^8 leucocytes) Mean ± SD	Overall	116·3±37·0	127·2±39·6	115·9±56·6	121·6±29·2
	Summer	111·0±24·4	115·5±33·4	117·9±51·1	119·6±25·6
	Winter	120·8±44·9	136·8±44·0	113·5±62·8	123·8±32·8

TABLE IV

Haematological measurements on samples taken on day of delivery

	Haematological values Mean ± SD			
	White cell count $(\times 10^{9}/l)$	Red cell count $(\times^{12}/l)$	Haemoglobin (g/dl)	Packed cell volume (l/l)
Summer	12·9 ±3·57	3·99±0·43	12·57±1·35	0·359±0·034
Winter	10·17±3·15	4·12±0·38	12·8 ±1·15	0·382±0·034
Total	11·38±3·36	4·06±0·41	12·68±1·25	0·371±0·034

on regression analysis of percentage change between late pregnancy and post partum.

An attempt was made to correlate in multigravidae the late pregnancy and first day post partum values for leucocyte ascorbic acid with previous reproductive performance. Of the 32 women with leucocyte ascorbic acid values less than 100 nmol/10⁸ leucocytes, 9 (28·1 per cent) had a history of pregnancy wastage which was no different to the figure of 29·2 per cent in the group with leucocyte ascorbic acid values greater than 100 nmol/10⁸ leucocytes. A total of 23 women had a history of pregnancy wastage and in this group, which had a leucocyte ascorbic acid value (mean±SD) of 115·8±34·8 nmol/10⁸ leucocytes, 9 (39·1 per cent) had leucocyte ascorbic acid values less than 100 nmol/10⁸ leucocytes. These figures were not significantly different to those obtained from the group of 57 women with no previous history of pregnancy wastage and a leucocyte ascorbic acid value of 123·5±43·5 with 40·4 per cent of values below 100 nmol/10⁸ leucocytes. The low number of previous stillbirths precluded conclusions about an association with leucocyte ascorbic acid values. There were no stillbirths in the present group of pregnancies.

DISCUSSION

Two facts were clearly shown by our work. Firstly, there was a marked depression of leucocyte ascorbic acid concentrations on the first day post partum. Secondly, in the latter stage of pregnancy, a relatively high proportion of our women were below the lower limit of normal for non-pregnant women (Denson and Bowers, 1961) and indeed the mean ascorbic acid concentration for the whole population was very much less than the values found by other workers. Loh *et al* (1974) give a value (mean±SD) of 193±62 nmol/10⁸ leucocytes in a group of non-pregnant women aged between 22 and 45 years. Indeed, leucocyte ascorbic acid concentrations are greater in the female than the male (Andrews and Brook, 1968; Dodds, 1969). This and various other factors suggest that women handle ascorbic acid differently from men (Loh, 1972). There are, for instance, certain changes which might be associated with changes in steroid concentrations and these have even been observed during the menstrual cycle with a peak of plasma ascorbic acid concentration at ovulation. Associated with this it has been noted that subjects ingesting oral contraceptives have lowered plasma ascorbic acid concentrations (Rivers and Devine, 1972; Briggs and Briggs, 1972). Indeed as part of this difference it has been concluded that metabolic readjustments can be made in both women and female guinea pigs to partially compensate for depressed levels of vitamin C by its synthesis (Wilson, 1974).

Allowance was made for factors which might have been associated with these depressions of vitamin C. Wilson (1974) showed that there was circadian cycle in ascorbic acid concentrations and so samples were taken at a similar time each day. It was thought that changes in platelet, or leucocyte counts might have caused some changes in the vitamin C concentration, but the results do not support this idea.

Depression of leucocyte ascorbic acid has been observed after viral infection (Hume and Weyers, 1973) or with stress (Chalopin *et al*,

1966) and that this is associated with neutrophilia. Similarly, oral contraceptive use lowers ascorbic acid concentrations (Rivers and Devine, 1972) and causes neutrophilia (Cruickshank and Alexander, 1970). The neutrophil fraction of the white blood cells has about half the concentration of ascorbic acid found in lymphocytes (B. D. Vallance, 1976, personal communication) and in addition granulocytosis shows a positive correlation with plasma cortisol concentrations (Hume *et al*, 1972). In the latter stages of pregnancy there is a two- to three-fold rise in cortisol concentrations (Cope, 1965) and at the same time there is a positively related leucocytosis due principally to an increase in the neutrophil count (Cruickshank, 1970). Although no correlation could be shown between leucocyte ascorbic acid concentrations and leucocyte counts in this study, it is possible that the changes observed were due (in part at least) to these changes in cortisol concentrations and the neutrophilia. An alternative explanation could be found in a redistribution of ascorbic acid from the white cells to the plasma and thence to maternal and fetal tissues in the latter stages of pregnancy.

It is probable that the most important factor associated with the initially depressed vitamin C levels is dietary, this being borne out by the significant differences in the mean ascorbic acid concentrations between the social classes. A second feature will presumably be related to the added requirements of blood-borne nutrients during pregnancy which will inevitably lower the concentrations of such nutrients. In such circumstances, the use of non-pregnancy norms is inadvisable and would be better replaced by specific standards for pregnancy. It is true to say, however, that the range of values observed in this study parallels our own observations of concentrations of leucocyte ascorbic acid in normal non-pregnant women.

It is surprising that we found no seasonal difference; this may reflect the present high nutritional status of the population in general which is no longer bounded by climatic considerations. Similarly, no change was observed in the different age groups studied. This is not surprising because of the relative lack of numbers and the narrowness of the range, which did not contain older women. Previous studies have shown depressed leucocyte ascorbic acid concentrations in geriatric populations (Wilson, 1974).

The numbers in this study are insufficient to define any specific health hazards that might be associated with the lowered ascorbic acid concentrations. The studies of River and Devine (1975), however, must be kept in mind in making such a statement. They clearly showed an improvement in reproductive performance in guinea pigs supplemented with ascorbic acid and this suggests that it is probable that some subclinical changes may well be taking place in humans. In addition, it is clear that lowered vitamin C saturation can significantly lower the rate of drug metabolism (Zannoni *et al*, 1972; Beattie and Sherlock, 1976) and ethanol oxidation (Krasner *et al*, 1974) and may therefore influence the essential metabolism of endogenous substances such as hormones and steroids in the latter stages of pregnancy.

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TRANSFER OF GLUTAMIC ACID ACROSS THE HUMAN PLACENTA PERFUSED *IN VITRO*

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Summary

Transfer of amino acids was studied with an *in vitro* perfusion technique of human placenta in which independant maternal and fetal circulations were established. When the fetal perfusate was recirculated, a concentration gradient was established with fetal concentrations of alanine, glycine and lysine about 1·5 times higher than maternal. In this system, the placenta could actively transfer 4·1 mM per day of each of these amino acids. Glutamic acid and aspartic acid were progressively removed from the fetal circulation so that fetal concentrations fell below maternal. The exchange rates of L- and D-glutamic acid were compared, the latter providing a measure of simple diffusion. The ratio of L:D exchange rates was 0·64 from the maternal to the fetal circulation and 0·41 in the reverse direction, indicating a more rapid bidirectional exchange of the D-isomer. Simultaneously measured exchange rates of leucine gave a L:D ratio of 1·73 and 1·00 respectively, indicating active transfer of L-leucine towards the fetus. While for L-glutamic acid, transfer across the placenta was even lower than expected on the basis of simple diffusion, uptake into the tissue was high. Of the amount presented to the placenta, 49 per cent was taken up, of which only 10 per cent passed to the other side. The rest was retained and presumably metabolized. For L-leucine, the uptake was only 16 per cent of which 75 per cent was transferred. The placenta serves as an effective barrier to the transfer of glutamic acid by rapidly metabolizing this amino acid. It is postulated that maternal glutamine, which is efficiently transferred across the placenta, contributes its amid-N_2 to the fetal synthesis of purines and pyrimidines and that the resulting surplus of glutamic acid is returned to the placenta which removes it from the circulation and metabolizes it.

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THE placenta must obtain its own nutritional requirements from the maternal circulation as well as those nutrients that are destined for transfer to the fetus.

Amino acids are transferred across the placenta from the mother to the fetus against a concentration gradient. With an *in vitro* perfusion technique for human placental tissue we have clearly shown that this is due to an active stereospecific placental transport system which functions, unidirectionally, from the maternal to the fetal side (Schneider *et al*, 1969 and 1978).

Glutamic and aspartic acid appear to be notable exceptions in that there is no net transfer of these amino acids across the placenta. *In vivo* studies in the rat and subhuman primate have demonstrated very poor placental transfer; injection of radioactive glutamic acid into pregnant animals was followed by the appearance of radioactive metabolites in the fetal circulation but not glutamic acid itself (Dierks-Ventling *et al*, 1971; Stegink *et al*, 1975). Incubation of human placental slices resulted in very rapid uptake with the development of strikingly high intracellular gradients (Schneider and Dancis, 1974).

In contrast to other amino acids, the concentration of glutamic acid in umbilical venous blood of human infants is lower than in the umbilical artery (Rooth and Nilson, 1964; Young and Prenton, 1969) indicating that there is no net uptake of glutamic acid by the fetus. Instead, the fetus produces an excess of glutamic acid which is removed by the placenta.

The mechanism is unknown by which the placenta acts as a barrier to the transfer of maternal glutamic acid to the fetus and also effectively removes glutamic acid from the fetal circulation. It appeared that these provocative problems might be approached with the technique of *in vitro* perfusion of the human placenta.

METHODS

Perfusion technique

The technique of dual perfusion of the human placental cotyledon has been published in detail (Schneider *et al*, 1972) and therefore only a brief description will be presented here. Fresh placentae from uncomplicated term pregnancies were obtained immediately after either vaginal or abdominal delivery. Both sides were carefully inspected and a suitable cotyledon was identified. The artery and vein supplying the cotelydon were identified on the fetal surface of the placenta, dissected, and fine polyethylene catheters were inserted and fixed by a 00 silk ligature. The perfusion of the fetal vascular tree was started immediately with oxygenated Earle's buffered salt solution. The cotyledon was then turned upside down and fixed in a perfusion chamber filled with buffer solution. The decidual plate assumed a white colour over the area which was perfused from the fetal side, indicating an efficient rinsing of the fetal capillaries. Two fine glass cannulas were inserted into the intervillous space (IVS) by penetrating the decidual plate over the light coloured area and the IVS was perfused through these cannulas. The perfusion fluid returned through venous openings in the decidual plate and collected in the upper part of the perfusion chamber from where it eventually drained through an overflow. The perfusion chamber, the two circuits and fluid reservoirs were mounted in a temperature controlled box at 37°C. Flow rates, pressures, PO_2, PCO_2 and pH were monitored in both circuits throughout the experiment. Samples could be taken separately from maternal and fetal inflow as well as outflow.

Five placentae were perfused with an open maternal circuit, which means that perfusate returning from the IVS was collected and not recirculated. The fetal circuit was closed in that a fixed volume of perfusate was recirculated through the capillary network of the fetal villi. Five different amino acids were added to the maternal and fetal perfusates in equal concentration of 0·25 mM each. Periodically, samples were taken from the fetal circuit for analysis of amino acid concentration. Because of the open maternal circuit, concentrations on the maternal side of the membrane did not alter throughout the experiment which lasted up to two hours.

In a second set of experiments which was used to measure exchange rates of D- and L-isomers of amino acids, maternal and fetal circuits were kept open. L- and D-isomers of leucine and glutamic acid were added to the maternal and fetal perfusates in equal concentrations (0·1 mM). Trace amounts of each isomer differentially labelled with ^{3}H or ^{14}C were added to the maternal or fetal perfusate.

Analytical methods

Non-radioactive amino acids were analyzed with a Phoenix Microanalyzer (model PKU). The samples were deproteinized with picric acid (Stein and Moore, 1954). Two buffer systems were used: 0·2 M Na^+, 0·05 M citrate, titrated to pH 3·05 (Hamilton, 1963), for the elution of the acid, neutral and branched-chain amino acids and 0·35 N Na^+ buffer, at pH 6·48, for the aromatic and basic amino acids (Seely *et al*, 1969). Radioactive amino acids were identified by paper chromatography. Five ml perfusate was mixed with an equal volume of 10 per cent perchloric acid for deproteinization. After centrifugation, the pH of the supernatant was adjusted to 7·0 with KOH and again centrifuged. The supernatant was applied to Dowex 50 (H^+), the resin was washed with water and the amino acids eluted with 2N NH_4OH. The eluate was taken down to about 1 ml in a Buchner flask evaporator and then blown to dryness in a small centrifuge tube, under nitrogen at 37°C. The residue was dissolved in 50 μl water and submitted to paper chromatography in butanol: acetic acid:water (12:3:5). Standard amino acids were chromatographed concurrently and located by development with ninhydrin. The parallel bands containing the radioactive amino acids were cut into small strips, and transferred to a scintillation vial with 3 ml water. The amino acids were eluted by shaking overnight in an incubator at 35°C. The strips were removed and the water evaporated under nitrogen. The residue was redissolved in 0·5 ml water and Diotol added. The exchange rates of L- and D-isomers were measured simultaneously by use of the double label, with a two-channel (^{3}H:^{14}C) scintillation counter.

Radioactivity in perfused tissue was analyzed in the same fashion after extracting 5 g of tissue with 5 ml of 10 per cent perchloric acid.

Materials

Non-labelled amino acids were purchased from Schwartz-Mann, and radioactive amino acids were obtained from New England Nuclear except for ^{14}C-D-glutamic acid which came from International Chemical and Nuclear Corporation. All radioactive amino acids were uniformly labelled with either ^{14}C or ^{3}H and were tested for purity by paper chromatography.

RESULTS

In experiments using the closed fetal circuit, the development of transplacental gradients for different amino acids was expressed as the ratio of concentration in the fetal perfusate over maternal substrate level. The results of one experiment are presented graphically in Figure 1. For alanine, lysine and glycine the movement from the maternal to the fetal side exceeded that in the reverse direction resulting in net transfer. At equilibrium the concentration on the fetal side was significantly above the maternal level.

In contrast, the fetal concentration of aspartic acid and glutamic acid declined and after 90 minutes of perfusion the two had almost completely disappeared from the fetal perfusate. Since maternal levels for these two—as for all five amino acids—remained constant during the entire experiment, a reverse transplacental gradient with the maternal level higher than the fetal developed for the two amino acids. The amino acid chromatogram of the serial samples from the fetal circuit showed a new peak which

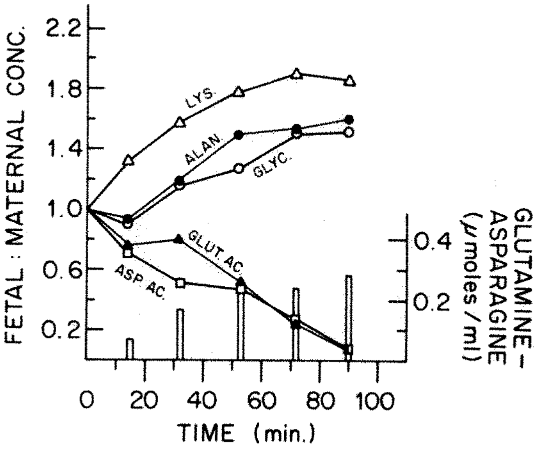

FIG. 1

Transplacental gradients. Five amino acids were added in equimolar concentrations, 0·25 mM, to maternal and fetal perfusates establishing a ratio of 1·0 at zero time. The maternal circuit was kept open, maintaining constant amino acid levels and the fetal circuit was closed permitting the fetal amino acid concentration to equilibrate against the maternal.

increased in size in subsequent samples. The elution time of this peak was identical with that of glutamine-asparagine in the system used. No glutamine or asparagine has been added to the maternal or fetal perfusates. Sodium citrate, which was used as buffer for the elution of the amino acids, does not separate the two amides.

Five experiments of this type were performed. Table I presents the mean of the transplacental gradients as measured under steady state conditions for the five amino acids.

TABLE I

Transplacental gradients

	Ratio of fetal: maternal concentrations (Mean ± SE)
Alanine	1·56 ± 0·11
Glycine	1·48 ± 0·09
Lysine	1·62 ± 0·10
Aspartic acid	0·13 ± 0·04
Glutamic acid	0·14 ± 0·03

Amino acids were added in equimolar concentrations to maternal and fetal perfusate. The maternal circulation was kept open, while the fetal perfusate was recirculated. After 90 minutes, concentrations in the two perfusates were determined. The results represent the mean of five experiments.

To further elucidate this difference in behaviour among the amino acids, the relative exchange rates for the stereo-isomers of leucine and glutamic acid were determined in both directions across the placenta. Three experiments each were performed to measure the exchange rates from maternal to fetal and fetal to maternal sides. After separately calculating the exchange rate for the L- and D-isomers, the ratios of these rates were determined. The results are summarized in Table II. The exchange of L-leucine from maternal to the fetal side was clearly at a faster rate than that for D-leucine as shown by a ratio of 1·73. In the reverse direction the mean of the ratios was 1·0 indicating no difference in ratios of exchange from fetal to maternal side for L- and D-leucine. In contrast, the ratios for the exchange rates of L- and D-glutamic acid were less than 1·0 in both directions which indicates that D-glutamic acid was transferred more efficiently across the placenta than the L-isomer.

Within the placenta, L-glutamic acid was found in higher concentration than D-glutamic acid, while no consistant difference was seen between L- and D-leucine.

TABLE II

Ratio of exchange rates of L and D isomers in three experiments

A. Maternal to fetal exchange rates				
	Transplacental		Placental	
Experiment	Glutamic acid L:D ratio	Leucine L:D ratio	Glutamic acid L:D ratio	Leucine L:D ratio
1	0·71	1·38	0·96	1·04
2	0·51	2·48	1·37	1·12
3	0·69	1·33	1·33	1·09
Mean	0·64	1·73	1·22	1·06
B. Fetal to maternal exchange rates				
1	0·56	1·04	1·30	1·10
2	0·19	0·86	1·50	1·00
3	0·49	1·11	0·95	0·99
Mean	0·41	1·00	1·25	1·03

L and D isomers of glutamic acid and leucine, differentially labelled with either ^{14}C or ^{3}H, were added to the maternal perfusate (A) or fetal perfusate (B). The ratio of their exchange rates (L:D isomer) into the placenta (placental exchange) and across the placenta (transplacental exchange) were calculated from the appropriate ^{3}H:^{14}C ratios (see text).

TABLE III

Comparison of placental uptake and placental transfer

	L-glutamic acid		D-glutamic acid		L-leucine		D-leucine	
	Uptake	Transfer*	Uptake	Transfer*	Uptake	Transfer*	Uptake	Transfer
	81·4	9·9	12·0	110·8	19·3	86·5	12·5	128·0
	25·5	10·2	11·0	125·5	16·3	58·8	8·1	138·3
	39·8	8·8	8·5	84·7	11·9	79·8	6·0	141·7
Mean	48·9	9·6	10·5	107·0	15·8	75·0	8·9	136·0

* Transfer is presented as per cent of uptake.

$$\text{Placental uptake (per cent)} = \frac{FA - FV}{FA} \times 100$$

$$\text{Placental transfer (per cent)} = \frac{MV \times QM}{FA \times QF} \times 100$$

Where MV = Maternal vein concentration; FA = Fetal artery concentration; FV = Fetal vein concentration; QM = Maternal flow rate; QF = Fetal flow rate.

The efficiency of extraction of substrate from the perfusate by the placenta (placental uptake) was measured and compared to transfer through the placenta. This study was performed from the fetal to the maternal side only. L and D isomers of glutamic acid and leucine differentially labelled with ^{3}H and ^{14}C were added to the fetal reservoir. Placental uptake was calculated from the arterio-venous difference and flow rate on the fetal side and was expressed as per cent of the arterial inflow of the amino acid. The latter was calculated from the arterial concentration and the flow rate. Transfer through the placenta was derived from the concentration in the venous outflow of the IVS and the maternal flow rate and was also expressed as per cent of uptake (see also Table III).

The results of three experiments each for glutamic acid and leucine are given in Table III. The behaviour of the two D-amino acids was quite similar. The mean placental uptake for D-glutamic acid was 10·5 per cent and for D-leucine 8·9 per cent of the arterial inflow. Placental transfer closely approximated to uptake, i.e. all the amino acid taken up was transferred. The slightly higher transfer than uptake noted in some of the experiments with D-amino acids probably resulted from a small bulkflow from the fetal to maternal side which was due to a higher intravascular pressure in the fetal circuit. This bulkflow did not affect the arterio-venous difference in the fetal circuit and therefore did not enter into the calculations of the placental uptake. But it did increase the recorded results for transfer.

Placental uptake of L-glutamic acid was more efficient than that of L-leucine (48 per cent versus 15·8 per cent of arterial inflow) but only 10 per cent of the uptake of L-glutamic acid from the fetal side of the placenta could be retrieved on the maternal side versus 75 per cent for L-leucine.

In the open fetal—open maternal circuit experiments, paper chromatography showed no significant conversion of L-glutamic acid during transfer through the placenta in either direction.

DISCUSSION

The technique of *in vitro* perfusion of a small area of the human placenta is particularly suited to answer some of the questions related to its function. It permits the study of transfer and metabolism without the confusing features of maternal and fetal metabolism. It further represents the only method currently available for examining the mechanisms of human placental transfer. In view of considerable species differences, methods which will provide informations on human placental metabolism and transport characteristics are badly needed.

The initial experimental design that was used for the study of transplacental amino acid transport was the open maternal—closed fetal circuit. This resembles the *in vivo* situation in that maternal substrate is supplied to the placenta at relatively constant concentrations in liberal amounts. What is observed is the placental capacity for controlling concentrations in the comparatively small fetal pool represented by the recirculating fetal perfusate.

Three of the five chosen amino acids behaved as expected. There was net transfer of alanine, glycine and lysine and the system reached a steady state at a transplacental gradient of around 1·5 for the ratio of fetal over maternal concentration. Transfer was against a gradient and therefore represented active transport. The rate of transfer could be estimated from the initial rate of increase of the concentration. As seen in Figure 1 before the steady state was reached the slope was quite similar for alanine, glycine and lysine. With a fixed volume of fetal perfusate of 50 ml and a mean weight of the perfused cotyledon of 20 g we estimated transfer of 2·8 μM per minute for a placenta of 450 g. This could provide a term fetus with 4·1 mM of each of the three amino acids per day. Mean umbilical uptake of various amino acids by the sheep fetus has been measured with the chronic catheter preparation (Lemons *et al*, 1976). The uptake for alanine was between 5 and 6 mM/day/kg fetal weight, a rate which will of course include a substantial component of fetal utilization. Precise data are not available for the human.

Contrasting with the behaviour of alanine, lysine and glycine, the fetal concentrations of glutamic and aspartic acid fell below maternal levels and continued to decline during the course of the perfusion (Fig. 1). These observations are consistant with observations in human infants at birth where umbilical venous concentrations are less than those in the artery indicating a loss of the two dicarboxylic amino acids during passage through the placenta (Rooth and Nilson, 1964). In the sheep, no transfer from the maternal circulation into the umbilical vein was observed (Lemons *et al*, 1976). In the present experiments, fetal glutamic acid was not replaced from the maternal perfusate.

While our *in vitro* observations on the regulation of fetal glutamic acid levels by the human placenta fit well with data from the sheep and the umbilical blood concentrations in infants, they are in apparent contrast to those described for the guinea pig. *In situ* perfusion of the fetal circuit of the placenta in a closed system resulted in a continuous increase in the glutamic acid concentration until the fetal perfusate almost equalled the placental tissue level (Hill and Young, 1973). This seems to be another important example of a species difference in placental transport characteristics.

The clearance of glutamic acid from the fetal system by the placenta could be achieved by different mechanisms. It could be accomplished by the transfer of glutamic acid across the placenta from the fetal to the maternal side. Another possibility is uptake into the placenta with storage and/or metabolism and conversion into other compounds. Support for the latter explanation was gained from the appearance of glutamine in the fetal circuit during the course of the perfusion. Active placental conversion of glutamic acid into glutamine which is released into the fetal circuit has been described in the rat (Dierks-Venting *et al*, 1971). As previously mentioned, the column chromatography system did not separate glutamine from asparagine, so that it is possible that both amides were synthesized from the respective amino acids.

In order to answer some of the questions related to the mechanism by which the placenta removes glutamic acid from the fetal circuit, experiments with differently labelled D- and L-glutamic acid and D- and L-leucine were performed, using equimolar concentrations in both circulations which were kept open (not recirculated) so that substrate concentrations were maintained constant. Radioactive tracers were added to either the maternal or fetal circulation. Under these conditions the transfer of the radioactive amino acid is a measure of the exchange rate. The basic premise in these studies was that exchange of the D-amino acid indicates diffusional exchange and that augmentation of the exchange rate for the L-isomer implied active transport mechanisms. This reasoning has been applied successfully to the study of placental transport of histidine by Page *et al* (1957) and of leucine by ourselves (Schneider *et al*, 1969 and 1978).

The results with leucine were consistent with previous experience and expectations. The exchange rate of L-leucine exceeded that of the D-isomer towards the fetus but the two were equivalent in the reverse direction (Table II). The L:D ratio of exchange rates clearly indicated an active transport system towards the fetus which was responsible for establishing the fetal-maternal gradient observed *in vitro* (Schneider *et al*, 1969) and *in vivo*.

The results with glutamic acid were surprising. The exchange rates in both directions were higher for D-glutamic acid than for the L-isomer (Table II). It appeared unlikely that this amino acid was unique in having a D-stereospecific transport system. Furthermore, using the slice technique, we have shown that L-glutamic acid is actively taken up and concentrated intracellularly by placental tissue (Schneider and Dancis, 1974). In the present studies, the placenta at the end of the perfusion had a higher concentration of L-glutamic acid (Table II).

The relation of placental uptake to placental transfer was studied with D- and L-glutamic acid and again with D- and L-leucine as a representative of an amino acid with fairly well studied placental transport characteristics. Placental uptake was deliberately studied from the fetal side since it seemed to be the mechanism by which the placenta regulated fetal glutamic acid levels.

In these experiments, L-glutamic acid was taken up by the placenta at a higher rate than L-leucine but while most of the L-leucine was exchanged across the membrane and appeared on the other side, very little of the L-glutamic acid was found on the maternal side (75 per cent versus only 10 per cent). Although the placental uptake of L-glutamic acid was only shown from the fetal side it is likely that it is also actively absorbed from the maternal blood.

The interesting conclusion to be drawn from these studies is that the placenta acts as a metabolic barrier to the transfer of glutamic acid. The amino acid is efficiently taken up from the fetal circulation by the placenta but very little is released into the maternal circulation. The amount retained within the placenta accounts for relatively little of the uptake so that the rest must be presumed to be metabolized. Identification of the major metabolic pathways of glutamic acid in placental tissue will be the subject of a future study.

There are still a number of important questions which remain unanswered. Since the glutamic acid which is required for fetal protein synthesis is not received from the mother, it must be formed by the fetus. The fetal production of glutamic acid actually exceeds its own requirements and the surplus is transferred to the placenta.

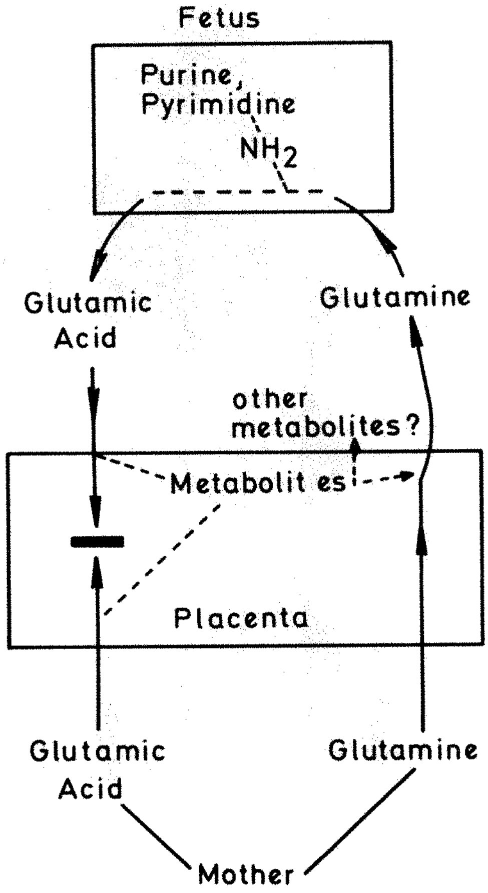

FIG. 2

Placental role in glutamine-glutamic acid metabolism. Maternal glutamine is transferred to the fetus where the amide nitrogen is donated to purine and pyrimidine synthesis. The resulting glutamic acid is used for protein synthesis and the excess is returned to the placenta where it is metabolized. The placenta also serves as a 'metabolic barrier' to maternal glutamic acid.

One possible source of fetal glutamic acid is by transamination of α-ketoglutaric acid. The conversion of α-ketoglutaric acid to glutamic acid could reduce fetal CO_2 production as suggested by Rooth and Nilson (1964). The production of glutamic acid via transamination may also provide a mechanism for the excretion of fetal nitrogen which could act to supplement an inefficient urea cycle in the fetus (Lemons *et al*, 1976).

Maternal glutamine may also be a major source of glutamic acid. Glutamine is efficiently transferred across the placenta (Schneider *et al*, 1978) and there is a high rate of uptake by the fetus (Lemons *et al*, 1976; Yamamoto *et al*, 1974). The high level of glutaminase activity in fetal tissue (McGee *et al*, 1971) would make the amide nitrogen available for the synthesis of purines and pyrimidines, and release glutamic acid. The great bulk of nucleic acids are synthesized by the fetus from simple precursors (Shambaugh *et al*, 1974) since there is no significant transfer from the mother (Dancis and Balis, 1954). A high uptake of glutamine was also shown for tumours, another type of rapidly growing tissue (Yamamoto *et al*, 1974). These postulated relationships are summarized in Figure 2.

According to the scheme, the primary role of placental metabolism of glutamic acid as it relates to the fetal economy is to remove it from the fetal circulation and prevent potentially toxic accumulations. In the process, the dicarboxylic acids may contribute to the energy requirements of the placenta as has been suggested in a parallel situation in intestinal absorption (Windmueller and Spaeth, 1975 and 1976). Nutrient metabolites of glutamic acid such as lactic acid and glucose could be released to the fetus as has been demonstrated in the subhuman primate (Stegink *et al*, 1975). Glutamic acid may also be converted to glutamine and released to the fetus as occurred in the closed fetal experiments (Fig. 1). The quantitative significance of these nutrients to the fetus remains to be determined. They may contribute relatively little when compared to the amount of nutrients obtained directly from the maternal circulation.

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THE SYNTHESIS OF IMMUNOREACTIVE PROLACTIN BY DECIDUA-CHORION

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Summary

Normal term human decidua-chorion, amnion and placenta were examined for possible synthesis of prolactin (PRL) by organ culture of tissue fragments in medium M199. Before incubation, higher PRL concentrations were found in decidua-chorion (27·7±5·7 ng/mg protein; mean±SE) and amnion (19·7±6·9 ng/mg protein) than in placenta (3·1±0·5 ng/mg protein; $p < 0{\cdot}005$). Incubated decidua-chorion released significantly more PRL into culture medium than did amnion or placenta. After 72 hours culture, the PRL concentration of decidua-chorion remained high (17·9±6·4 ng/mg protein); that in amnion (2·3±1·9 ng/mg protein) fell to approach that of placenta (1·4±1·0 ng/mg protein). In further studies, fragments of decidua-chorion were incubated in medium RPMI 1640 without or with puromycin (0·04 mM) or cycloheximide (0·1 mM). A decrease in medium PRL concentration was observed within four hours of adding these inhibitors of protein synthesis. At four hours, the PRL content of decidua-chorion cultured in puromycin (12·4±3·8 ng/mg protein) or cycloheximide (11·9±2·9 ng/mg protein) was lower than tissue incubated in medium alone (25·5±4·1 ng/mg protein; $p < 0{\cdot}05$). Comparable differences in tissue content and medium concentration were also seen at 24 and 72 hours. We conclude that human decidua-chorion synthesizes a protein immunologically similar to PRL and that this tissue may represent the principal source of amniotic fluid PRL.

THE amniotic fluid of primates contains very high concentrations of prolactin (PRL) (Tyson *et al*, 1972; Badawi *et al*, 1973; Clements *et al*, 1977) but its origins are unknown. It does not seem to come from the maternal circulation as isotopically labelled PRL injected into the pregnant rhesus monkey shows little or no transfer to the amniotic fluid (Friesen *et al*, 1972; Josimovich *et al*, 1974). A second possible source of PRL is via passage of fetal pituitary PRL through the kidney (Josimovich *et al*, 1974; Chochinov *et al*, 1976). However, human amniotic fluid concentrations of this hormone remain unchanged despite bromocriptine suppression of maternal and fetal plasma PRL values throughout pregnancy (del Pozo *et al*, 1977), suggesting an independent origin. A third source may be the decidua, fetal membranes or placenta. PRL has been localized by immunofluorescence to human amnion (Healy *et al*, 1977) and is released on incubation of human decidua and amnion-chorion (Riddick and Kusmik, 1977) but not of placenta (Friesen *et al*, 1972). However, it is unclear from these reports whether this hormone was attached to and then released from cell surfaces or receptors, or locally manufactured. The aim of this study was to investigate the hypothesis that these tissues may synthesize PRL.

MATERIAL AND METHODS

Preparation of tissue

Placentae and amniotic membranes were collected from term human pregnancies delivered electively by Caesarean section for maternal indications. The tissues were immediately cooled in ice and rinsed clean of blood and amniotic fluid with phosphate buffered saline (pH 7·4). Amnion was separated from decidua-chorion by careful peeling and fine dissection and each tissue and pieces of placental cotyledon were washed for three 10-minute intervals at 4°C in oxygenated medium 199 (Commonwealth Serum Laboratories, Australia) at pH 7·4 to remove residual amniotic fluid PRL.

Organ culture

Within 90 minutes of delivery, 10 to 20 mg pieces of amnion, decidua-chorion or placenta were organ cultured on 0·8 μm Millipore filter paper situated on wire grids in 1·5 ml of medium 199, pH 7·4. The cultures were maintained for up to 72 hours in 5 per cent CO_2 in oxygen at 37°C, with media changes at 4, 24 and

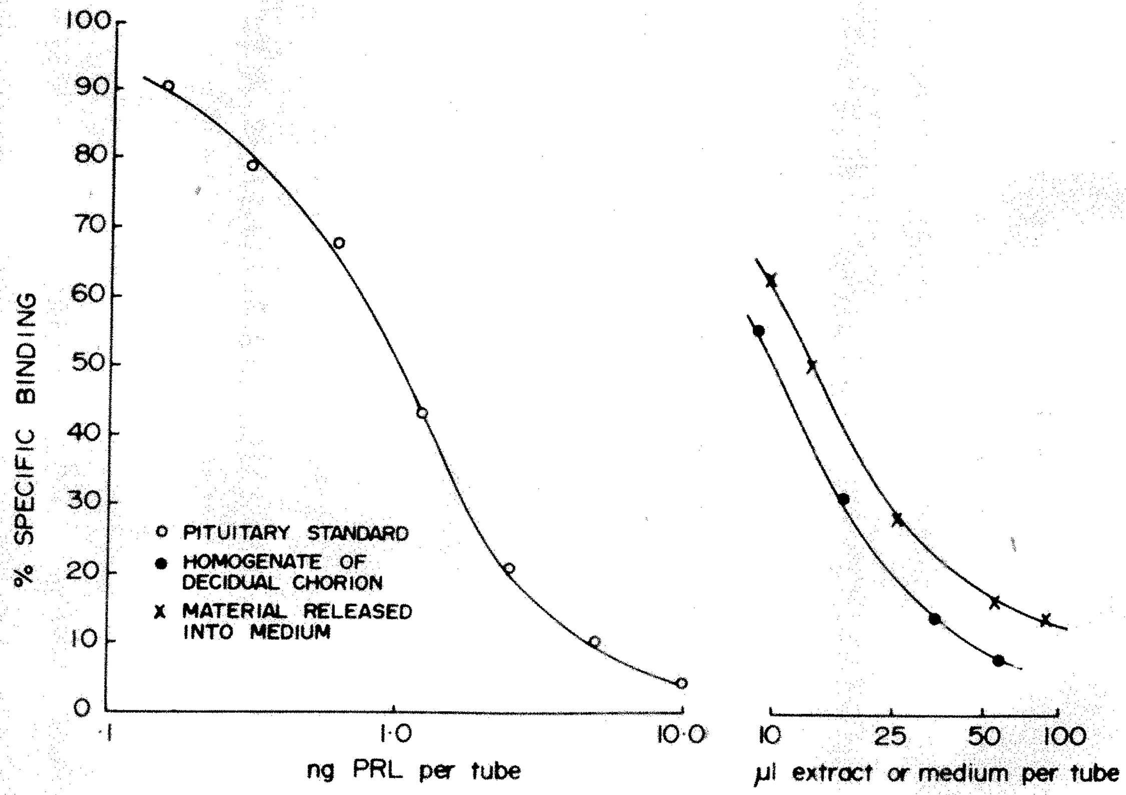

FIG. 1

Parallelism of dilutions of representative samples of decidua-chorion tissue extract and culture medium with that of pituitary PRL in radioimmunoassay.

72 hours, and PRL content estimated by radioimmunoassay as previously described (Healy and Burger, 1977) using materials supplied by the National Institutes of Health, Bethesda, Maryland. The antiserum did not cross-react with chorionic gonadotrophin, chorionic somatomammotrophin or prostaglandins E_1 and E_2. Media alone did not react in this radioimmunoassay and all samples from each patient were processed at the same time in a single assay which had a mean sensitivity of 0·5 ng/ml.

In subsequent experiments, fragments of decidua-chorion were cultured in medium RPMI 1640 (CSL, Australia) either alone, under the conditions described above, or in medium with added puromycin (0·04 mM) or cycloheximide (0·1 mM) for up to 120 hours. RPMI 1640 was chosen as a more nutritive medium for organ culture studies. Media were changed and assayed as previously described.

Histology and extraction

Tissue samples were taken before and at various stages of culture and fixed in Bouin's fluid for conventional histology. Parallel samples were extracted by sonication in 0·1 M NH_4HCO_3 using a Branson Sonifier Model B–30 at 0°C, centrifuged (30 minutes at 3300 *g*), and the supernatants assayed for their PRL content. A small sample was removed for protein determination (Lowry *et al*, 1951). Statistical analysis between groups was made using Student's 't' test and data expressed as the mean ± SEM.

Gel filtration

A column of G75 Sephadex, 25 × 1·5 cm, was prepared and equilibrated. The void volume was calculated using a 0·2 per cent solution of blue dextran and the column calibrated by use of ^{125}I–PRL (VLS3 preparation of pituitary PRL supplied by the NIAMDD), MW 21 000 and ^{125}I to determine the salt volume. Term amniotic fluid and aliquots of decidua-chorion homogenate after centrifugation and organ culture medium, known to contain high PRL concentrations, were eluted through this column. The fractions were analysed by PRL radioimmunoassay.

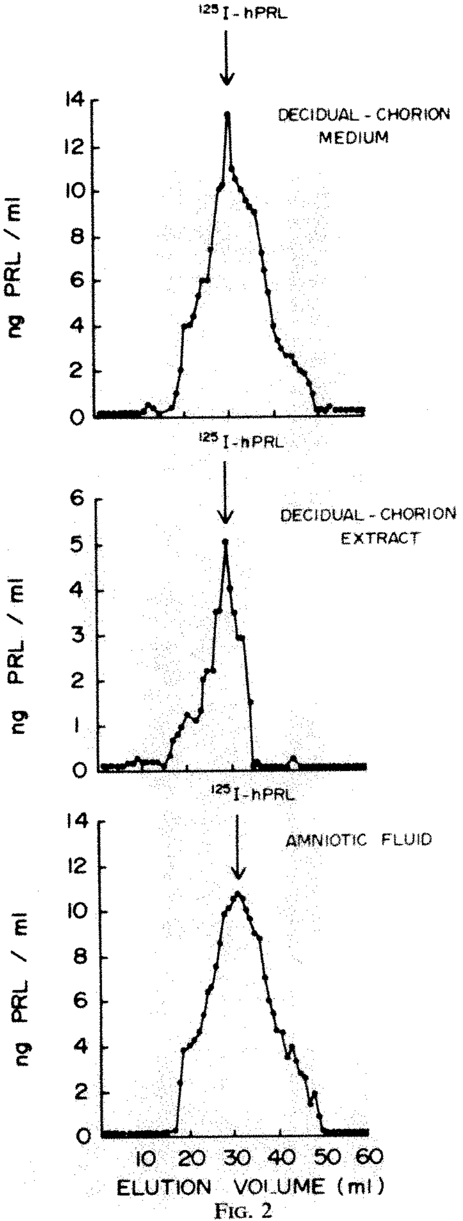

FIG. 2

Sephadex G–75 gel filtration (25 × 1·5 cm) of decidua-chorion extract and organ culture media and amniotic fluid. Each sample was applied in a volume of 1·0 ml and eluted with 0·08 M barbitone buffer, pH 8·6, containing 1 per cent bovine serum albumin (void volume 15 ml, total column volume 56 ml). One ml fractions were collected and the activity of the fractions was quantified by radioimmunoassay. Approximately 80 per cent of the PRL in the decidua-chorion medium, 87 per cent of the PRL in the decidual-chorion extract and 75 per cent of the PRL in amniotic fluid eluted in the position corresponding to the ^{125}I-hPRL pituitary monomer.

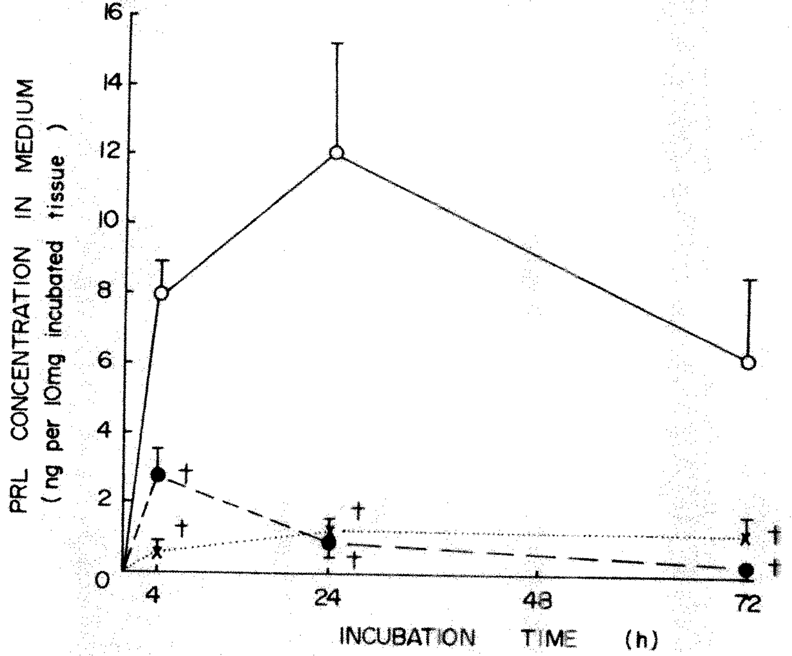

FIG. 3

PRL concentrations in culture media (ng per 10 mg incubated tissue) after incubating decidua-chorion (○———○), amnion (● — — — ●) and placenta (×.........×) in medium 199. The values are means of triplicate cultures from three patients. †p < ·005 comparing decidua-chorion with amnion or placenta.

RESULTS

Immunoreactive PRL in decidua-chorion

Extracts of decidua-chorion and organ culture medium after decidua-chorion incubation were found to contain a material which exhibited parallel inhibition with the PRL standard curve (Fig. 1). Sephadex G-75 gel filtration chromatography of selected samples of the supernatant of decidua-chorion homogenate and medium after incubation was compared with the results of amniotic fluid gel filtration. A typical chromatogram is depicted in Figure 2. The major peak of immunoreactivity in all samples eluted in the region of ^{125}I pituitary PRL monomer.

PRL released on incubation of decidua-chorion, amnion and placenta

Figure 3 shows that incubated decidua-chorion released significantly more PRL into the medium than did amnion or placenta. This difference was observed at four hours and continued for at least 72 hours. At four hours, more PRL was released by amnion than by placental cultures. Histology demonstrated only minimal degeneration in tissue pieces after 72 hours culture.

Effect of incubation upon tissue PRL content

Sonication of tissues before incubation revealed that decidua-chorion and amnion con-

TABLE I

Comparison of the PRL content (ng/mg protein) of amnion, decidua-chorion and placenta from term pregnancies before and after 72 hours incubation in medium 199

Tissue	Before incubation	72 hour incubation
Amnion	19·7±6·9	2·3±1·9†
Decidua-chorion	27·7±5·7	17·9±6·4
Placenta	3·1±0·5†	1·4±1·0†

The values are the means of quadruplicate cultures from seven patients.

† p <0·005 comparing decidua-chorion with amnion or placenta.

tained higher PRL concentrations than did placenta (Table I). After 72 hours incubation the PRL content of the decidua-chorion, but not the amnion, was maintained at close to the initial value and comparison of the tissues at 72 hours showed that the decidua-chorion contained significantly higher PRL concentrations than either amnion or placenta.

TABLE II

PRL yield before and after 72 hours incubation of decidua-chorion (ng/10 mg wet weight)

Patient	Tissue content		Cumulative media content	Yield at 72 hours
	0 hours	72 hours		
1	6·8	3·6	59·5	63·1
2	1·0	5·1	30·0	35·1
3	4·4	1·3	32·0	33·3
4	2·7	1·4	34·5	35·9
5	0·6	2·3	21·3	23·6

The values are the means of triplicate cultures.

Total PRL yield from decidua-chorion incubation

Table II summarizes data from five patients when both media PRL content and tissue PRL concentration are expressed as ng PRL per 10 mg incubated tissue. The total PRL yield at 72 hours was calculated as the sum of the cumulative media content plus the tissue PRL content at 72 hours. Comparison of the yield at 72 hours with the tissue content before incubation shows a 7 to 35-fold increase in PRL over that time, suggesting hormone synthesis by this tissue.

Effects of puromycin and cycloheximide upon decidua-chorion PRL

Figure 4 compares the effect of inhibitors of protein synthesis on PRL release from decidua-chorion. Four hours after adding puromycin (0·04 mM) or cycloheximide (0·1 mM), a difference in the PRL concentrations in the media was apparent. After 24 hours incubation, a marked reduction in PRL release had occurred in the presence of these inhibitors and this was maintained at 72 and 120 hours. At 120 hours, histological examination of tissues cultured in the presence of puromycin or cycloheximide

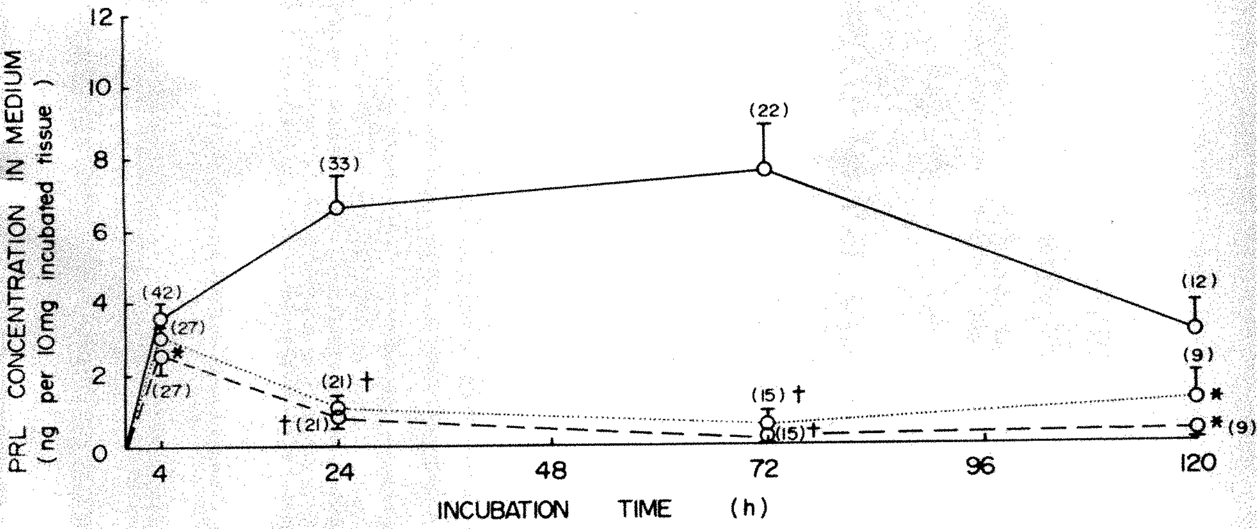

FIG. 4

PRL concentrations in culture media (ng per 10 mg incubated tissue) after incubating decidua-chorion in medium RPMI 1640 (O———O), medium RPMI 1640 and 0·04 mM puromycin (O — — — — O) and medium RPMI 1640 and 0·1 mM cycloheximide (O · · · · O). The values are the means of observations from three patients, the number of cultures being indicated in parentheses. $^{*}p < 0{\cdot}05$; $^{\dagger}p < 0{\cdot}005$ comparing control medium with puromycin or cycloheximide cultures.

TABLE III

Comparison of the PRL content (ng/mg protein) of decidua-chorion cultured in medium RPMI 1640 without and with puromycin or cycloheximide

Medium	Incubation time (hours) 4	24	72	120
RPMI 1640	25·5±4·1	24·6±7·3	11·3±3·2	0
RPMI 1640, Puromycin 0·04 mM	12·4±3·8*	6·9±4·3*	3·2±2·5*	0
RPMI 1640, Cycloheximide 0·1 mM	11·9±2·9†	8·2±4·8*	0†	0

The values are the means of quadruplicate cultures from three patients.
* $p < 0\cdot05$; † $p < 0\cdot025$ comparing medium without or with inhibitors.

showed only focal areas of moderate cell degeneration.

Table III describes the PRL content of the decidua-chorion cultured in the presence of medium RPMI 1640 without or with puromycin or cycloheximide. At four hours there was a lower tissue concentration of PRL in the presence of either inhibitor. At 24 and 72 hours, the tissue concentration of PRL in the presence of puromycin or cycloheximide were further reduced compared with control cultures and reflected the low PRL values found in the media. PRL was not detected in any tissue extract after 120 hours incubation.

DISCUSSION

These studies demonstrate that decidua-chorion synthesizes a protein immunologically and chromatographically similar to human PRL. Total PRL yield from this tissue after 72 hours incubation is 7 to 35-fold greater than the initial PRL concentration in decidua-chorion. Incubation in the presence of specific inhibitors of protein synthesis markedly reduces PRL levels in the decidua-chorion, consistent with synthesis of immunoreactive PRL by this tissue. Histological examination of control and test tissues showed only focal areas of cell degeneration at 120 hours incubation. This excludes cell necrosis or autolysis, with release of proteolytic enzymes, as an explanation for the results obtained.

The cell type in decidua-chorion responsible for PRL synthesis has not been identified and could be fetal or maternal in origin. Decidual cells are a possible source (Riddick and Kusmik, 1977) and decidua is capable of protein synthesis (Miller, 1973). PRL synthesis by a normal extra-pituitary tissue is unique and factors governing its production and secretion in decidua-chorion during human pregnancy are unknown. *In vitro*, optimal conditions for PRL synthesis by decidua-chorion are still to be determined. Examination of Figure 3 shows that if PRL secretion was expressed per unit time, one would see a rapid decline in the release of PRL into the culture medium over time. This would be similar to what has been described for the release of human chorionic somatomammotrophin from placental organ culture (Franchimont *et al*, 1972), the decline being possibly associated with the artificial conditions of culture.

Although the concentration of PRL in decidua-chorion found in this study is small compared with the pituitary (Hwang *et al*, 1973), the amount of hormone manufactured may be comparable since the total mass of secretory decidual-chorion tissue is large in pregnancy. Moreover, amniotic fluid at 12 to 16 weeks of pregnancy contains much higher concentrations of immunoreactive prolactin than does amniotic fluid at term (Clements *et al*, 1977), suggesting the amount of hormone produced may be greater in the second trimester than in term tissue.

Decidual-chorionic PRL is strategically placed for mediating possible local effects of this hormone, such as regulation of water and electrolyte fluxes across the amniotic membranes (Manku *et al*, 1975; Josimovich *et al*, 1977;

Riddick and Kusmik, 1977) and inhibition of myometrial contractility (Mugambi *et al*, 1974). Abnormalities of decidual-chorionic PRL synthesis may provide insights into mechanisms important in the pathogenesis of oligohydramnios or polyhydramnios and prematurity.

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EFFECT OF INTRAUTERINE CONTRACEPTIVE DEVICE ON UTERINE HAEMOSTASIS: A MORPHOLOGICAL STUDY

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Summary

The effect of the intrauterine contraceptive device (IUCD) on uterine haemostasis was studied at various stages of the menstrual cycle in a series of 46 patients by light- and electron-microscopy and by following the distribution of an infusion of ^{51}Cr-labelled autologous platelets. The endometrium in contact with the IUCD in the majority of cases showed grooving with atrophy and mild chronic inflammation in the surrounding tissues. The adjacent stroma also showed increased vascularity and occasional foci of haemorrhage but the increased blood loss associated with the presence of the IUCD could not be attributed to mechanical erosion or disruption of stromal blood vessels by the device. During menstruation the presence of an IUCD does not appear to inhibit the formation of fibrin/platelet thrombi although both in control and IUCD patients there was a striking paucity of platelet/fibrin thrombi in circumstances where their formation should be enhanced. In contrast to other workers we have not observed that gaps or breaks in the endothelial lining of endometrial blood vessels occur with any greater frequency in patients fitted with an IUCD. The principal mechanism by which uterine haemostasis is achieved remains to be established.

MENORRHAGIA and intermenstrual bleeding are frequent side-effects associated with the intra-uterine contraceptive device (IUCD). Although fibrinolytic mechanisms have been studied in patients with an IUCD who complained of menorrhagia (Rybo, 1966; Larsson *et al*, 1974; Kasonde and Bonnar, 1976) most previous investigations on the effects of IUCDs on the uterus have been concerned with their role as contraceptive devices (Hall *et al*, 1965; Willson *et al*, 1965; Bonney *et al*, 1966; Buckle and Barnett, 1966; Ancla *et al*, 1967; Rozin *et al*, 1967; Tamada *et al*, 1967; Moyer and Mishell, 1971; González-Angulo *et al*, 1973; González-Angulo and Aznar-Ramos, 1976; Hsu *et al*, 1976; Ober, 1977). The role of fibrin and platelets in the haemostatic control of menstrual blood loss is poorly understood and little is known of the effects of IUCD on uterine haemostasis.

We report here the results of a morphological study by light- and electron-microscopy on the effects of IUCD on uterine vasculature and haemostasis. The distribution of labelled autologous platelets has been followed throughout the menstrual cycle with the aid of autoradiography and scintillation counting.

METHODS

Patients. We studied 46 patients at hysterectomy, 23 had had an IUCD inserted and 23 had not. The duration of menorrhagia was similar in the two groups 0·8±0·5 years and 0·9±0·6 years respectively. The diagnosis of menorrhagia was based on a detailed menstrual history. The indications for hysterectomy and the types of IUCD used are shown in Table I. The stage of the cycle at hysterectomy, assessed by a combination of histological and clinical data, is shown in Table II. With the exception of six patients (three in each group) all received an infusion of autologous platelets labelled with ^{51}Cr in the week before hysterectomy.

TABLE I

Clinical details of patients studied

Clinical data	Patients without IUCD	Patients with IUCD*
	(n = 23)	(n = 23)
Age (years (Mean ± SD)	37·1±6·5	37·7±5·1
Indication for hysterectomy:		
Menorrhagia	19	18
Prolapse	4	5
Duration menorrhagia (years)	0·9±0·6	0·8±0·5
Duration IUCD (years)	—	5·0±2·9 (range: 0·3–11·0)

* Patients with: Lippes Loop = 18; Saf-T-coil = 2; Dalkon Shield = 2; Copper 7 = 1.

Sampling procedure. Adjacent blocks were removed from the uterus immediately after hysterectomy for light- and electron-microscopy, scintillation counting and autoradiography. The endometrium and the subjacent myometrium in contact with the IUCD (usually fundus) and at some distance from it (usually lateral wall) were excised. In patients without an IUCD similar specimens were taken from two standard areas in the fundus and lateral wall.

Light-microscopy. The blocks for light-microscopy were fixed in formalin or Zenker's fixative, processed routinely through paraffin and 5 μm sections were stained with haematoxylin and eosin (HE), Giemsa (for platelets) and martius yellow, scarlet red and soluble blue (MSB; for fibrin).

Immunofluorescence. Thirty-eight uteri were studied by immunofluorescence using acetone-fixed frozen sections of fresh material stored in liquid nitrogen until use. A fluorescein isothiocyanate conjugate (FITC)-labelled anti-human fibrinogen preparation (Wellcome Laboratories) was employed at a dilution that abolished non-specific background staining (usually 1:10 or 1:20 dilution). Preparations of FITC-labelled anti-human IgG and anti-human albumin were used as controls.

Electron-microscopy. Blocks were sectioned into approximately millimetre cubes (40 per uterus), fixed in phosphate buffered 5 per cent glutaraldehyde, post-fixed in 2 per cent osmium tetroxide and embedded in Epon (Luft, 1961). Large thin (500 μm) blocks were flat embedded in an orientation that allowed the selection of large semi-thin (250 nm) sections of the entire thickness of the endometrium. These preparations were used for counting vascular gaps. Semi-thin sections were stained with methylene blue/Azur II to facilitate the final selection of areas for ultra-thin sectioning (Richardson *et al*, 1960). Ultra-thin sections (50 nm) were stained with 8 per cent uranyl acetate and 0·5 per cent lead citrate. The ultrastructural evaluation was performed with a Philips EM 300 and an average of 20 fields per uterus were photographed at magnifications of 1 to 30×10^3.

Autoradiography. Autoradiography was performed on semi-thin sections, coated with Kodak AR-10 stripping film and exposed for four weeks. After development at 20°C for eight minutes with Kodak D19b developer, the autoradiographs were stained with Azur II. Grains were counted from at least ten fields per section at $\times 10^3$ magnification.

Scintillation counting. Scintillation counting of whole uteri was performed in a shielded counter (J. & P. Engineering) with 2π geometry. Samples of endometrium and myometrium adjacent to areas used for microscopy were digested in 5 ml of molar hyamine hydroxide in methanol and counted in a γ-counter (Nuclear Enterprises).

RESULTS

Morphological effects of IUCD

In 15 out of 23 patients with an IUCD it was possible to identify a groove or depression where the device had been in contact with the endometrial surface, the edge of which was rounded or thrown up into irregular folds (Fig. 1). In the remaining patients there was no obvious distortion of the endometrial surface. The epithelium lining the grooves was atrophic and partly eroded and in nearly one-third of patients contained foci of squamous metaplasia. The glands in the surrounding endometrium were atrophic and appeared not to have responded to cyclical hormonal stimulation. The associated stromal tissues were condensed and fibrotic and showed increased vascularity with a light infiltrate of polymorphs and lymphocytes (Fig. 2). Rarely, there was a more generalized low-grade chronic endometritis involving most of the endometrium. The dilated capillary blood vessels surrounding the grooves often came close to the epithelial surface but open communication with the uterine cavity was never seen. Occasional vessels had ruptured producing small subepithelial foci of stromal haemorrhage but these did not communicate with the surface. Although changes were most marked in women in whom the device had been present for several years they did not always correlate with the duration of use and in one instance where an IUCD had been present for 11 years no pathological changes were seen. The subjacent myometrium and the endometrium away from contact with the device showed no abnormalities.

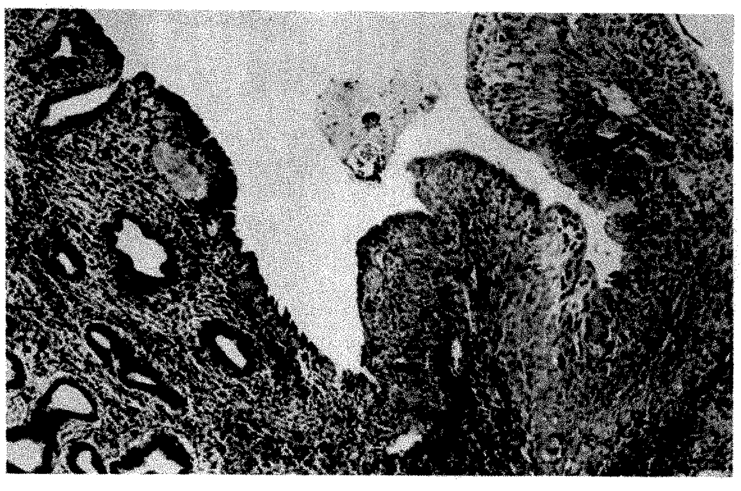

FIG. 1

Endometrial groove with polypoid edge produced by IUCD. The adjacent endometrium is atrophic and the lining epithelium is partly eroded. (HE, ×72·5).

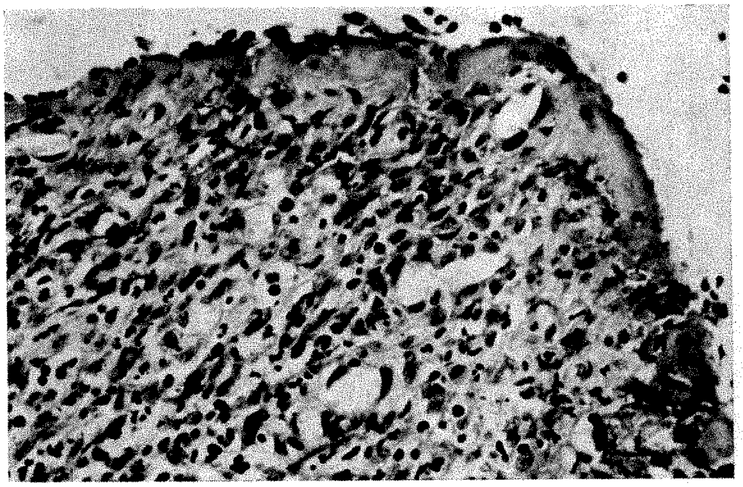

FIG. 2

Endometrium adjacent to groove showing erosion of surface epithelium with fibrosis and increased vascularity of the stroma; the stroma also contains a light infiltrate of lymphocytes and polymorphs. (HE, ×290).

In patients without the IUCD, no significant histological abnormalities were seen in the endometrium or myometrium and the glandular elements showed a regular response to cyclical hormonal stimulation.

Fibrin and platelets in uterine haemostasis

Of the 46 uteri examined only 13 (five from patients without and eight from patients with an IUCD) were considered to be in the menstrual phase when assessed by a combination of histological and clinical criteria. The small number of patients involved permit only tentative conclusions concerning the role of fibrin and platelets in haemostasis during menstruation. The results are summarized in Table II.

TABLE II

Effect of IUCD on fibrin content of endometrial blood vessels

		No. patients with vessel involvement				
		Degree of vessel involvement (per cent)				
No. patients	Menstrual cycle phase	0	1	2–10	11–19	20
Without IUCD						
8	Follicular	6	1	0	1	0
10	Luteal	4	3	1	1	1
5	Menstrual	0	1	2	1	1
With IUCD						
9	Follicular	6	2	0	1	0
6	Luteal	1	2	2	1	0
8	Menstrual	1	3	0	3	1

Intravascular fibrin (as demonstrated by positive MSB staining) was found in all five uteri from patients without IUCD and in 7 of the 8 uteri from patients with IUCDs, although only a small proportion of the endometrial vessels were involved. Attempts were made to quantify this and the number of fibrin-positive vessels was scored on a histological scale ranging from a minute trace (one per cent vessels) to a moderate amount (20 per cent vessels). The results show (Table II) that the presence or absence of an IUCD had no significant influence on the number of fibrin-containing vessels in the endometrium. Similarly the distribution of fibrin-containing vessels within the endometrium was the same for both groups; positive vessels which were mainly of the venous type, tended to occur in small clusters with a greater frequency in the compact and spongy layers than the basal layer (Fig. 3). The earliest stage after commencement of vaginal bleeding when fibrin could be detected was three hours in patients without an IUCD and five hours in patients with IUCDs. Fibrin deposition was quantitatively greater during the early stage of menstruation (0 to 48 hours) than at the later stage and in both groups the latest stage that intravascular fibrin could be demonstrated was three days after vaginal bleeding commenced when there were only trace amounts present.

Immunofluorescence studies confirmed the paucity of fibrin deposits in menstruating endometrium and good correlation was obtained between amounts of fibrin demonstrated by MSB staining and immunofluorescence techniques. In addition immunofluorescence revealed the presence of trace amounts of fibrin in the interstitium of the endometrial stroma in both groups.

Light-microscopy revealed little evidence of

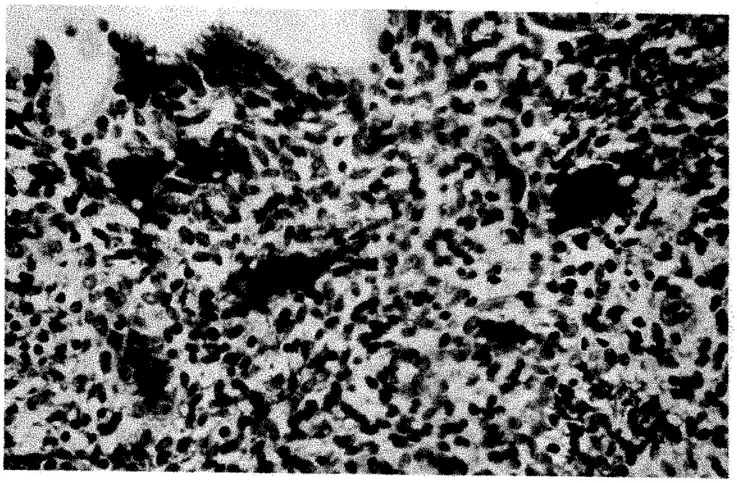

FIG. 3

Cluster of venous channels staining positively for fibrin (black) in superficial layer of endometrium. (MSB, ×290).

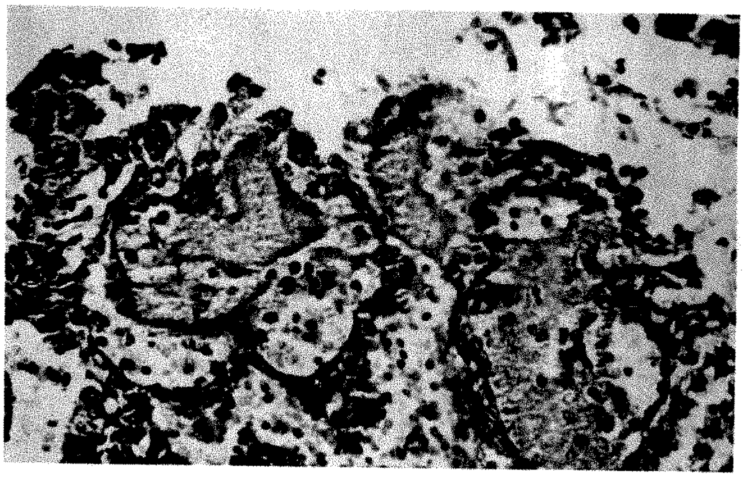

FIG. 4

Menstrual fragments on surface of endometrium containing fibrin/platelet thrombi within disrupted vascular channels. (MSB, ×290).

platelets in the thrombosed vessels of unshed endometrium. However, in both groups small mixed platelet/fibrin thrombi could occasionally be seen within detached and degenerate menstrual fragments on the endometrial surface (Fig. 4).

Ultrastructural studies

Platelet and fibrin thrombi were found in endometrial blood vessels in 5 out of 5 patients without IUCDs and in 5 out of 8 with IUCDs during menstruation. They were rarely found in non-menstruating patients. Only a small proportion of vessels in menstruating endometrium contained thrombi confirming the light-microscopy and immunofluorescence findings. No significant ultrastructural differences could be detected between thrombi in the two groups of patients. In both groups, thrombi were composed of platelets and fibrin although occasional thrombi consisting of platelets or fibrin alone were seen. In most thrombi the platelet component predominated and consisted of a central core of electron-dense elements surrounded by distended and degranulated platelets and a narrow rim of fibrin (Fig. 5). The fibrin component was composed of fibrillar aggregates with normal electron-density and cross-banding characteristics. The vast majority of thrombi were located in disintegrating venous channels with only an occasional thrombus being found in a spiral arteriole. Occasional loose aggregates of platelets were found extravascularly in both groups amongst shed and degenerate menstrual fragments on the endometrial surface.

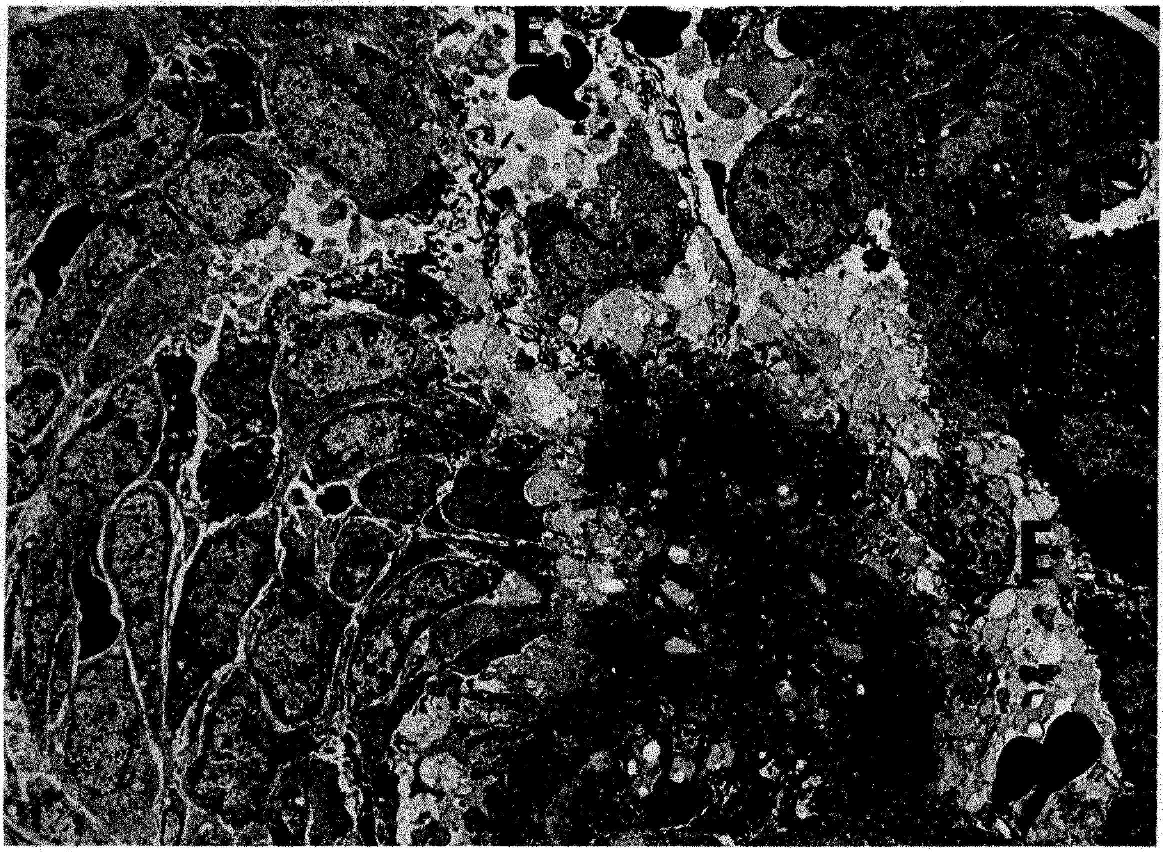

FIG. 5

Electron micrograph of platelet/fibrin thrombus (Th) within a disrupted blood vessel; the lining endothelial cells (E) show degenerative changes. Adjacent endometrial gland (G) and stromal cells (S) are also shown. (×2160).

The disintegration of small blood vessels is a common feature in menstruating endometrium and may be overlooked when the original outline of the vessel is disrupted. Indeed in some patients it was possible only to delineate the presence of vessels by their thrombotic contents (Fig. 5).

Interruptions or gaps in the wall of such vessels are commonly seen through which the formed elements of the blood can escape into the surrounding stroma (Fig. 6). Although such disintegration predominantly affected venous channels, in one patient without an IUCD, degeneration of the endothelium lining spiral arterioles was seen. This did not affect the muscular component of the wall and interruptions in the continuity of the vessel wall as in venous channels were not observed.

Less commonly 'gaps' were seen in vascular channels of capillary size that appeared to constitute true 'breaks' or 'fractures' of the thin cytoplasmic processes of endothelial cells away from tight junctions. These were found only in vessels with large and often irregular lumina that contained no erythrocytes and were lined by a thin endothelial layer surrounded by a poorly formed or absent basal lamina (Fig. 7). The appearance of these vessels corresponds to that of lymphatic channels. An attempt was made to quantitate the presence of endothelial gaps in selected patients (Table III). The results show that neither the presence or absence of an IUCD nor the physical contact with the device had any influence on the frequency of such gaps.

Spiral arterioles were occasionally demonstrated in both groups in which the lumen was reduced to a narrow slit and contained electron-dense plasma. The smooth muscle elements in the media of these vessels appeared contracted.

Labelled platelet studies

Whole-organ scintillation counts indicated that no uterus contained more than 0·12 per cent

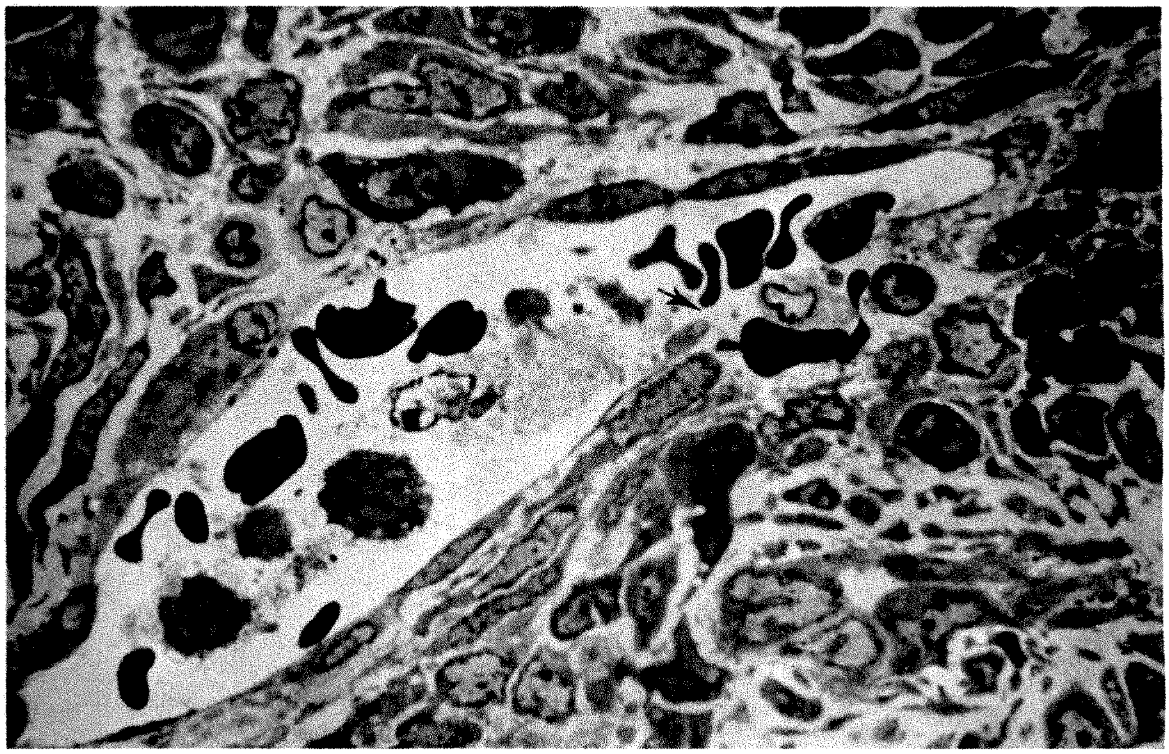

FIG. 6

Semi-thin section (250 nm) of menstrual endometrium showing gap (arrowed) in venule through which formed elements of blood escape into stroma. (Methylene blue/Azur II, × 1760).

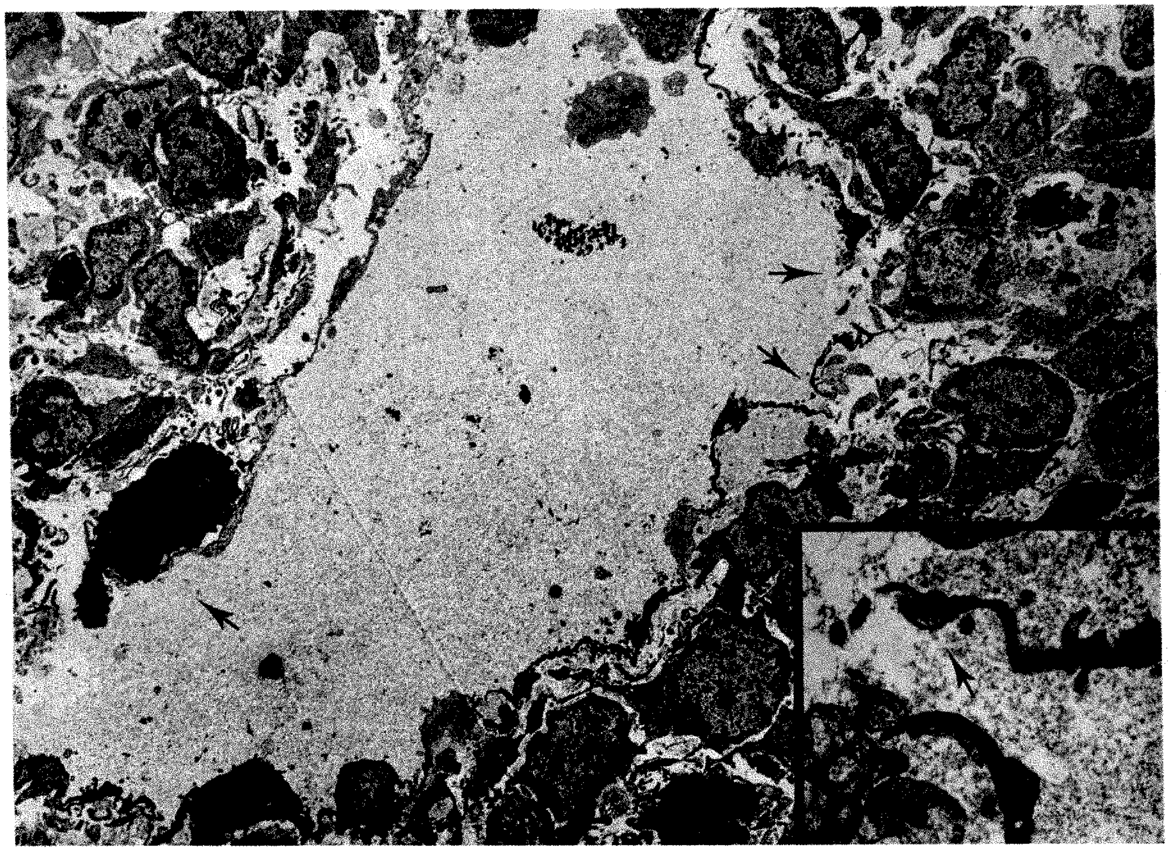

FIG. 7

Electron micrograph of endometrial lymphatic showing gaps (arrowed) through which, in one instance, a macrophage passes (bottom left). (×2160). INSET: detail of gap showing intact interendothelial junctions (arrowed). (×12800).

TABLE III

Effect of IUCD on number of 'gaps' in lymphatics in menstruating endometrium

Group	No. endometrial vessels assessed	Mean no. gaps per vessel	
		In contact with IUCD*	Away from IUCD†
Without IUCD	367	0·07	0·12
With IUCD	472	0·08	0·07

* Fundus in patients without IUCD.
† Lateral in patients without IUCD.

of the infused ^{51}Cr activity. The counts did not show any significant difference between the two groups of patients nor was there any correlation with the phase of the menstrual cycle.

Scintillation counts of tissue blocks (Table IV) revealed that activity was greater in the endometrium than in the myometrium in virtually all patients. The mean counts for 'in contact' endometrium from patients with IUCDs were not significantly different from the corresponding fundal samples in the others. In both groups the mean count for endometrium 'in contact' (fundal in patients without IUCD) was approximately half that of the 'away' endometrium (lateral in patients without IUCD).

In the autoradiographs grain counts were low. However, the ^{51}Cr level as determined by grain counting was higher in the endometrium away from contact with the IUCD as compared to areas in contact, with respective mean counts (±SD) of 15·3±8·4 and 11·0±6·8. No differences were observed in the grain counts

TABLE IV

Effect of IUCD on ^{51}Cr activity associated with endometrium and myometrium

Group	No. patients	^{51}Cr activity (c.p.s./g tissue) (Mean ± SD)			
		In contact with IUCD*		Away from IUCD†	
		Endometrium	Myometrium	Endometrium	Myometrium
Without IUCD	14	0·97±1·02	0·46±0·47	1·97±2·30	0·41±0·40
With IUCD	15	1·01±1·57	0·35±0·33	1·85±2·70	0·26±0·19

* Fundus in patients without IUCD.

† Lateral in patients without IUCD.

taken respectively over the endometrium in lateral (13·2±9·7) and fundal (12·5±7·4) positions in the uteri from patients without IUCD.

DISCUSSION

Our findings confirm previous studies of the morphological effects of IUCDs on the uterus. Grooves or indentations are produced in the endometrium where the device comes into contact with the surface and the adjacent tissues show atrophy and mild localized chronic inflammation. However, the extent of these changes varied in different patients indicating that there may be some movement of the device within the cavity. Where there were grooves the surrounding stroma showed increased vascularity and occasional foci of stromal haemorrhage. However, open communication of dilated vessels or areas of stromal haemorrhage with the uterine cavity was not demonstrated and although some erosion of the epithelium lining the grooves occurred this did not appear to give rise to intracavitary haemorrhage. Thus, simple mechanical erosion of superficial blood vessels in the endometrium is an unlikely explanation for the haemorrhage associated with the use of IUCDs.

The most striking finding in our study was the relative paucity of fibrin and platelet thrombi in the blood vessels of menstruating endometrium in both groups of patients. During menstruation it would appear that only a small proportion of vessels (1 to 20 per cent) contain fibrin/platelet thrombi in circumstances where there is considerable disruption of endometrial blood vessels and conditions ought to favour the formation of platelet/fibrin thrombi. These findings were confirmed by immunofluorescence studies using specific anti-sera for fibrin and by electron-microscopy. The presence of an IUCD apparently has no detrimental qualitative effects on the haemostatic processes and the scanty thrombi found in both groups of patients were similar. The patients without IUCD mostly suffered from menorrhagia and may not be strictly comparable with non-menorrhagic patients from the haemostatic point of view, although pre-operatively a large panel of haemostatic tests was normal in these patients. Furthermore, the small number of non-menorrhagic patients included in the study showed similar findings to the rest. An unexpected finding was the demonstration of fibrin-like material in the stromal tissues of late luteal and menstrual endometrium in both groups of patients. This suggests that a fibrin-like molecule may leak into the interstitial stroma prior to and during menstruation. The significance of this observation in relation to uterine haemostasis is uncertain.

The studies with radio-labelled platelets support the morphological findings and no uterus contained more than 0·12 per cent of the originally infused ^{51}Cr activity. The difference in levels of labelling observed did not correlate with the phase of the menstrual cycle nor distinguish between the two groups of patients. These findings are supported by the platelet survival data to be reported elsewhere (Rajah *et al*, in preparation). Calculated on a weight basis, considerably less of the platelet infusion was retained than would be expected of such a vascular organ as the uterus. Whilst a substantial proportion of the infused platelets may have been lost during the operative removal of the uterus, when considerable effusion of blood may occur, the low levels of activity retained are somewhat surprising. The reduction in ^{51}Cr activity in endometrium in contact with the device was confirmed both by scintillation counting and autoradiography and was found at all phases of the menstrual cycle. In view of the generally low level of platelet retention it is difficult to be certain whether this represents a disruption of the normal haemostatic mechanisms in the areas adjacent to the IUCD or not.

Whilst there is some preliminary evidence from our studies to suggest that platelets and fibrin may be more extensively involved in haemostasis during the first 24 hours of menstruation than at later stages, the findings indicate that a major mechanism in addition to platelet/fibrin thrombus formation must be involved in the haemostatic processes of menstruation. Our studies were largely carried out at the later stages of menstruation and the early phases could profitably be examined in more detail.

Others have reported the presence of gaps or breaks in the endothelial lining of endometrial blood vessels which, they claim, are more frequent in patients with IUCDs (Hohman *et al*, 1977). They also suggest that such gaps in conjunction with a delay in thrombus formation at sites of contact with IUCDs may be responsible for the excessive menstrual bleeding. However, in this study we have been unable to confirm the importance of vascular breaks as an explanation for the increased bleeding tendency of patients with IUCDs. The apparent disintegration and fracture of small blood vessel walls is a very common feature of menstruating endometrium. It is particularly evident during the early stages of menstruation when it can be easily overlooked if the original outlines of the vessel wall are disrupted. In these vessels, breaks or gaps through which the formed elements of the blood may escape, can be demonstrated with ease. However, we believe that this is a non-specific phenomenon allied to the disintegration of the endometrium as a whole during the process of menstruation and has no specific pathological significance.

Much less commonly true gaps or breaks are demonstrable in the endothelial lining of certain thin-walled vessels that are normally found in endometrium and which we consider to be lymphatics. The gaps in the endothelium of these vessels may result from dilatation of fenestrations that are normally present in the walls of lymphatic channels and when counted with the electron-microscope they were found with no greater frequency in patients with IUCDs than in controls. The presence of lymphatics in the endometrium is a feature that is not well documented and we feel that failure to appreciate their existence may well confuse ultrastructural studies of menstruating endometrium.

Thus at present the increased blood loss experienced by patients with IUCDs remains inadequately explained. It seems clear that it is not attributable to simple mechanical erosion or disruption of endometrial blood vessels by the device. Furthermore, we have been unable to convince ourselves that the endometrial vasculature sustains any significant additional structural damage. The presence of the IUCD appears not to interfere with the formation of platelet/fibrin thrombi during menstruation although thrombotic occlusion of endometrial vessels does not appear to be a major haemostatic phenomenon at least during the later stages of menstruation. The principal mechanism by which uterine haemostasis is achieved in normal and IUCD patients remains to be established.

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A RANDOMIZED DOUBLE-BLIND TRIAL OF TWO LOW DOSE COMBINED ORAL CONTRACEPTIVES

BY

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Summary

Fifty-five women using Loestrin–20 (20 μg ethinyl oestradiol and 1 mg norethisterone acetate) as an oral contraceptive have been compared with a like number using Microgynon–30 (30 μg ethinyl oestradiol and 150 μg levonorgestrel) in a randomized, double-blind trial. Despite the small sample size, the main finding in the trial is clear-cut; Loestrin–20 provides poor cycle control and is thus less acceptable as an oral contraceptive than Microgynon–30. Although there is also a suggestion that Loestrin–20 may be less effective than Microgynon–30, the difference in the accidental pregnancy rates is not statistically significant.

IN an endeavour to minimize the risks associated with oral contraception, manufacturers have, over the years, steadily reduced the dose of the oestrogen component of the pill. Preparations containing less than 50 μg oestrogen first became available in the United Kingdom in 1973 and have now captured more than half the market (Intercontinental Medical Statistics, 1978, personal communication). Most of these low dose products contain 30 μg oestrogen, but one, Loestrin–20, contains only 20 μg ethinyl oestradiol (in combination with 1 mg norethisterone acetate). In 1974, the Family Planning Association (FPA) was asked to evaluate the use-effectiveness and acceptability of Loestrin–20. Accordingly, it was decided to conduct a multicentre randomized double-blind trial in which the new product would be compared with a pill containing 30 μg ethinyl oestradiol and 150 μg levonorgestrel (Microgynon–30) which was already in widespread use in family planning clinics.

METHODS

Seven family planning clinics (some under FPA administration, others administered by Area Health Authorities) agreed to collaborate in the trial and enrolment of patients commenced in November 1974. Owing to the slow rate of recruitment, however, the number of participating clinics was later increased to 12. At each clinic, patients were allocated to the two products at random. Neither the clinic doctor nor the patients were told the composition of the pills involved, but they were informed that both the products were combined oral contraceptives containing no more than 50 μg oestrogen. This information was also given to each patient's general practitioner. The pills were supplied in pre-coded sealed envelopes containing standard unmarked 21 day blister packs.

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To be eligible for recruitment, volunteers had to meet all the normal FPA requirements for oral contraceptive treatment (see FPA Clinic Handbook) but, in addition, they had to be (i) aged 16 to 39 years and of child-bearing potential, (ii) having regular sexual intercourse, (iii) menstruating regularly, (iv) prepared to accept the possibility of a slight risk of involuntary pregnancy, and (v) sufficiently reliable to keep monthly diary charts and to return to the clinic for follow-up. Women who were lactating or with a recent history of intermenstrual bleeding or spotting were specifically excluded from the trial.

At the initial visit, a history was taken, weight and blood pressure were recorded and a gynaecological examination was made. Patients were then issued (i) with an envelope containing three months' supply of the appropriate pills, (ii) with written instructions about how to take them, and (iii) with a diary card on which to note the days pills were taken and the occurrence of any bleeding (requiring sanitary protection) or spotting (not requiring sanitary protection). Trial participants were advised to take the pills according to the standard 21 days on/7 days off regimen, commencing on day 5 of the next menstrual period, and using additional contraceptive precautions during the first 14 days of treatment. Follow-up visits were arranged at 3-monthly intervals.

A total of 133 patients was recruited between November 1974 and September 1976. Of these, 23 have been excluded from the analysis: 11 who had taken Loestrin–20 or Microgynon–30 before entering the trial, 7 who never commenced taking their pills, and 5 who did not return to the clinic after their initial visit and could not be traced. The present report thus concerns 110 patients, of whom 55 were admitted to each of the two treatment groups. All but 6 of these subjects (3 in each treatment group) were successfully followed either until they discontinued the trial pill or up to the study closure date (31st December, 1976).

RESULTS

The characteristics of the subjects in the two treatment groups are summarized in Table I. Although there are some minor differences, none approaches statistical significance and there is no suggestion that the randomisation procedure was in any way unsatisfactory.

Table II shows the *net* cumulative discontinuation rates per 100 women at selected ordinal months of use computed by the standard methods described by Tietze and Lewit (1974). *Gross* discontinuation rates were also calculated, and differences in these rates between the two treatment groups were tested for statistical significance by the log-rank method described by Azen *et al* (1977). It can be seen from Table II that women using Loestrin–20 discontinued treatment because of abnormal bleeding far more often than women using Microgynon–30 ($\chi_1^2 = 10\cdot 1$, $P < 0\cdot 01$) but that the rates of discontinuation for other reasons were closely similar in the two treatment groups. Of the 13 women who stopped taking Loestrin–20 because of abnormal bleeding, 6 complained of oligomenorrhoea or amenorrhoea while the other 7 complained of irregular bleeding. Of the 2 women who stopped taking Microgynon–30

TABLE I

Characteristics of the subjects in the two treatment groups on admission to the trial

Characteristic	Loestrin–20	Microgynon–30
Mean age (years)	25·3	26·1
Nulliparous (per cent)	45	35
Irregular periods (per cent)	13	13
Usual cycle length > 30 days (per cent)	11	20
Usual duration of bleeding > 7 days (per cent)	0	7
Using combined pill as last contraceptive (per cent)	65	62
Number of subjects in group	55	55

TABLE II

Net cumulative discontinuation rates per 100 women by reason for discontinuation at selected ordinal months of use

Reason for discontinuation	Treatment group	Ordinal months of use 4	8	12	Significance of difference*
Accidental pregnancy	L	1·8	4·1	4·1	NS
	M	0·0	1·9	1·9	
Abnormal bleeding	L	7·5	17·8	27·0	$\chi^2_{(1)} = 10\cdot1$
	M	3·7	3·7	3·7	P <0·01
Other side effects	L	12·9	17·0	20·0	NS
	M	9·3	17·5	20·6	
Planning pregnancy	L	1·9	6·0	11·8	NS
	M	1·9	4·1	7·2	
Other personal reasons	L	3·7	10·0	10·0	NS
	M	5·6	7·6	14·1	
Continuation rate	L	72·2	45·1	27·1	—
	M	79·4	65·2	52·4	
Woman-months of use (cumulative)	L	189	288	333	—
	M	191	331	400	

L = Loestrin–20 M = Microgynon–30

* Log rank method using gross rates (see Azen *et al*, 1977)

because of disturbances of bleeding, one complained that bleeding was irregular and the other that it was prolonged.

In analysing the data on bleeding patterns recorded on the diary cards, we defined 'menstrual bleeding' as *any* bleeding of sufficient severity to require sanitary protection, irrespective of whether it occurred on days when tablets were taken or during the 7 day tablet-free intervals. This, of course, implies that the terms 'breakthrough bleeding' or 'intermenstrual bleeding' are inapplicable to our analysis. On this basis, the overall distribution of menstrual cycle length in the two treatment groups is given in Table III. A few women in each of the groups failed to complete their diary cards properly and have, therefore, not contributed to the table, but this is unlikely to be of any consequence. The results shown in Table III indicate quite clearly that Loestrin–20 provides much less satisfactory cycle control than Microgynon–30; in view of these data the pattern of discontinuation of use of the two products shown in Table II is not surprising.

TABLE III

Distribution of cycle length in the two treatment groups

Cycle length (days)	Loestrin–20 No. of cycles	per cent	Microgynon–30 No. of cycles	per cent
–17	60	21·7	28	6·1
18–24	14	5·1	10	2·2
25–31	150	54·4	397	86·5
32–38	11	4·0	7	1·5
39–	41	14·8	17	3·7
Total	276	100·0	459	100·0

Table IV provides data on the duration of menstrual bleeding in the two treatment groups. Again, Loestrin–20 appears to be less satisfactory than Microgynon–30, its use being associated with an excess of both short (up to 3 days) and prolonged (7 or more days) episodes of bleeding. Table V summarizes the information on spotting, both that occurring immediately before or immediately after menstrual bleeding

TABLE IV

Distribution of duration of menstrual bleeding in the two treatment groups

Duration of menstrual bleeding (days)	Loestrin-20		Microgynon-30	
	No. of cycles	per cent	No. of cycles	per cent
1-3	143	51·8	187	40·7
4-6	110	39·9	258	56·3
7-9	21	7·6	12	2·6
10-	2	0·7	2	0·4
Total	276	100·0	459	100·0

TABLE V

Distribution of duration of spotting in the two treatment groups

Number of days of spotting	Loestrin-20		Microgynon-30	
	No. of cycles	per cent	No. of cycles	per cent
'Linked' to menstrual bleeding				
0	160	58·0	273	59·5
1-2	96	34·8	159	34·6
3-	20	7·2	27	5·9
Total	276	100·0	459	100·0
'Unlinked' to menstrual bleeding				
0	244	88·4	436	95·0
1-2	19	6·9	16	3·5
3-	13	4·7	7	1·5
Total	276	100·0	459	100·0

('linked' spotting) and that occurring at other times in the cycle ('unlinked' spotting). Differences between the treatment groups are small but, on balance, somewhat in favour of Microgynon-30.

We examined the nature of the 'other side effects' (depression, headache, nausea, etc.) leading to discontinuation of Loestrin-20 and Microgynon-30 (see Table II) but could discern no indication of any differences between the groups. It may be noted, however, that the one woman who developed a deep vein thrombosis during the trial did so during the fourth month of treatment with Microgynon-30. We also analysed the limited data available on body weight and blood pressure; no significant changes in either measurement occurred in either contraceptive group during the course of treatment.

Accidental pregnancies

Two women had accidental pregnancies while taking Loestrin-20, one during the second cycle and one during the seventh cycle. Neither woman admitted to having missed any pills, but both stated that they might have taken a pill several hours late during the cycle in which they conceived. Both women carried their pregnancy to term, and both gave birth to normal healthy infants (one male, one female).

The woman who became pregnant while taking Microgynon-30 did so during the fifth cycle. She claimed to have taken all her pills exactly in accordance with the instructions. The pregnancy was terminated at 8 weeks gestation.

One of the 23 women excluded from the analysis is also known to have had an accidental pregnancy (proven by two positive pregnancy tests) while taking Loestrin-20. The pregnancy occurred during the third cycle, was not associated with any irregularity in the taking of pills, and ended in a spontaneous abortion at 8 to 10 weeks gestation.

DISCUSSION

Despite the small sample size, the main finding in this clinical trial seems to be clear-cut: Loestrin-20 provides poor cycle control and, as a result, is a less acceptable contraceptive than Microgynon-30. There is also a suggestion that Loestrin-20 might be less effective than Microgynon-30, but the difference between the accidental pregnancy rates does not reach statistical significance.

Other published data about the efficacy and acceptability of Loestrin-20 seem to be limited to those described by Preston (1972). This author reported the results of a study in which the experience of 1218 subjects receiving Loestrin-20 who were followed for a total of 8284 cycles was compared with the experience of comparable numbers of subjects receiving different dosage combinations of ethinyloestradiol and norethisterone acetate. While Loestrin-20 was only a little less effective than the pills containing higher dosages of the constituent steroids, there were striking differences in the frequency of amenorrhoea and irregular bleeding in line with those observed in the present study. In a later paper, Preston (1974) up-dated the information on efficacy on the basis of larger numbers of subjects and cycles.

Whether there is a logical place in the contraceptive armamentarium for Loestrin–20 seems uncertain. If, however, the major cardiovascular adverse effects associated with the use of combined pills are attributable to the oestrogen component, we rather suspect that women who are unwilling to accept pills containing 30 μg oestrogen on the grounds of safety would do better to try a progestogen-only preparation than to adopt an ultra-low oestrogen dose pill like Loestrin–20.

ACKNOWLEDGEMENTS

We thank Parke-Davis and Co. for financial support and for supplying Loestrin–20 and Schering Chemicals Ltd. for supplying Microgynon–30. We also thank the doctors, nurses and lay staff at the participating clinics for their careful supervision of the study subjects.

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HYPERPROLACTINAEMIA IN A PATIENT WITH THE McCUNE-ALBRIGHT SYNDROME

BY

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Summary

A patient is presented with the syndrome of polyostotic fibrous dysplasia and precocious puberty (McCune–Albright Syndrome). In adult life she developed hyperprolactinaemia with galactorrhoea and amenorrhoea; there was also evidence of excessive secretion of growth hormone.

THE McCune-Albright syndrome consists of polyostotic fibrous dysplasia of bone, sexual precocity and pigmentation; the syndrome is known to be associated with a variety of endocrine disorders. We present the first report of a patient with this syndrome who developed hyperprolactinaemia.

CASE REPORT

The patient was 30 years old and presented with secondary amenorrhoea for eight months. Galactorrhoea had been noted intermittently for five years. There was a history of precocious puberty; she had menstruated at three- to six-monthly intervals from the age of 19 months; breast development occurred during infancy; she was considered tall as a child but stopped growing at the age of seven years (final height 140 cm). Her periods became more regular (monthly) at the age of 11 years. She had normal pregnancies at the ages of 18 and 20 years, and a stillbirth at 22. At the age of 25 bilateral pyosalpinges and a benign right ovarian cystadenoma were removed. At the age of 26 years the patient developed a blood-stained discharge from the right nipple and a lump in the right breast; the lump was excised and histology showed fibroadenosis. The patient had a previous history of pathological fractures of both arms between the ages of 15 and 25.

On examination she had asymmetry of the face, with prominence of the forehead, mild proptosis on the right, and enlargement of the right maxilla. The right humerus was bowed. Bilateral galactorrhoea was evident. She had a small soft diffuse goitre but was clinically euthyroid. Visual fields were normal. There was no recent enlargement of hands or feet and she did not appear grossly acromegalic. There was patchy pigmentation on the lips.

Skeletal X-rays showed changes of fibrous dysplasia affecting the skull, several bones of both upper and lower limbs, vertebrae and pelvis. The pituitary fossa was not enlarged on plain films or tomograms, but thickening of the skull base around this area was noted.

The basal serum prolactin concentration was 1824 mU/l (normal <360 mU/l) rising to

2868 mU/l two hours after 10 mg oral Maxolon. The basal sample for prolactin assay was taken at 1000 hours through a forearm vein cannula inserted one hour previously, with the patient resting. The serum thyroxine concentration was 107 nmol/l. Thyroid microsomal and thyroglobulin auto-antibodies were negative. Serum thyroid stimulating hormone levels were normal basally (2·7 mU/l) and at 20 minutes and 60 minutes after the intravenous injection of 200 μg of thyrotrophin-releasing-hormone (10·7 mU/l and 7·6 mU/l respectively).

Serum gonadotrophins rose following the intravenous injection of 100 μg of luteinizing-hormone-releasing-hormone: luteinizing hormone (LH) levels were 8·9, 12·4 and 13·4 U/l, and follicle stimulating hormone (FSH) levels were 4·5, 6·1 and 8·1 U/l, basally and at 20 minutes and 60 minutes respectively after the injection. After seven days on 200 mg oral clomiphene daily, serum LH levels rose from 5·3 to 11·5 U/l, and serum FSH levels rose from 2·3 to 5·1 U/l, but a plasma progesterone level later showed no evidence of ovulation having been induced.

Serum growth hormone levels failed to show normal suppression following 50 g of oral glucose, the blood samples being taken through an indwelling forearm vein cannula: the basal concentration of growth hormone was 21 mU/l and the levels were 23, 16, 17 and 20 mU/l at 30, 60, 90 and 120 minutes respectively after the glucose load. Glucose tolerance was normal.

Plasma cortisol levels and serum growth hormone levels rose normally during hypoglycaemia induced by an intravenous injection of soluble insulin (0·15 units/kg body weight).

Within two months of treatment with oral bromocriptine, 2·5 mg twice daily, the patient noted a return of menstruation and cessation of galactorrhoea; the serum prolactin levels, although still above normal, had been reduced to about half the basal level before treatment.

DISCUSSION

Polyostotic fibrous dysplasia is well known to be associated with sexual precocity (Albright *et al*, 1937). The occasional association of the McCune-Albright syndrome with other endocrine disorders, such as acromegaly, Cushing's syndrome, goitre and accelerated growth, has led to the suggestion that hypothalamic dysfunction underlies the condition (Hall and Warrick, 1972; DiGeorge, 1975). Although gynaecomastia has been reported at the time of the precocious puberty in males with the syndrome, hyperprolactinaemia with galactorrhoea and amenorrhoea, as seen in our patient, has not previously been reported.

It is uncertain whether our patient, whose growth hormone levels are raised, will ultimately develop frank acromegaly a diagnosis that will be made difficult by the existing facial changes due to fibrous dysplasia of the skull bones. It is also uncertain whether a small pituitary tumour is present, but the occurrence of hyperprolactinaemia lends further support to the hypothesis that hypothalamic dysfunction underlies the McCune-Albright syndrome.

ACKNOWLEDGEMENTS

We are indebted to the supra-regional assay service for hormone assays, to Dr H. G. Friesen for providing reagents for the prolactin assay, to Dr C. K. Warrick for his helpful comments about the patient's X-rays, and to the Northern Regional Health Authority for a grant in support of Dr Colman.

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METASTATIC BASAL CELL CARCINOMA OF THE VULVA
CASE REPORT

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Summary

A patient with basal cell carcinoma of the vulva with tumour in local lymph nodes is described.

BASAL cell carcinoma accounts for only 2 or 3 per cent of vulval cancers (Schueller, 1965). Previous publications have suggested that basal cell carcinoma of the vulva is a non-metastasizing neoplasm (Palladino *et al*, 1969; Breen *et al*, 1975; Cruz-Jimenez and Abell, 1975).

We now present a case report which contradicts this point of view.

CASE REPORT

A 71-year-old patient presented with a lump on the mons pubis which had been present for 14 years and had caused discomfort for one year. On examination there was an ulcerated hard mass on the mons pubis extending down to the prepuce of the clitoris. There was no palpable lymph node enlargement. Radiographs of the pelvis showed no evidence of metastases in the pelvic bones.

The lesion was completely excised. It had not invaded muscle or bone and complete skin cover of the operation site was obtained.

The specimen consisted of skin measuring 10 × 6·5 cm bearing an irregularly shaped ulcer with slightly raised rolled undermined edges. The ulcer measured 8 × 4·5 cm. The floor of the ulcer was roughened and white and its base formed by firm white tissue which extended about 0·5 cm into adjacent fat. Within the fat on the right side five firm white lymph nodes were found. Microscopy of the ulcer showed cells with large ovoid nuclei, scant cytoplasm and many mitotic figures in solid and adenoid arrangements with variable prominence of peripheral pallisading extending downwards through the dermis in a prominent fibroblastic and collageneous stroma (Fig. 1). The five lymph nodes from the subcutaneous fat contained metastatic tumour in a fibroblastic stroma (Figs. 2 and 3). Malignant cells were also present in subcapsular sinuses and efferent lymphatics. Multiple sections of the tissue beneath the main tumour mass and above the lymph nodes failed to show any direct extension.

DISCUSSION

This case report demonstrates the usual presentation of basal cell carcinoma of the vulva, there being a long history of vulval mass and discomfort (Palladino *et al*, 1969). In both skin and lymph nodes the typical desmoplastic stromal reaction associated with basal cell carcinoma was

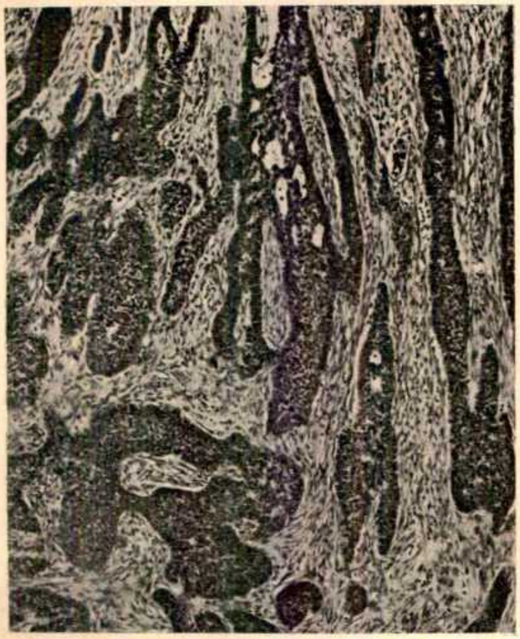

FIG. 1

Basal cell carcinoma of vulva composed of hyperchromatic cells in solid and adenoid arrangements with peripheral pallisading lying in a fibroblastic stroma (haematoxylin and eosin ×13).

present. The histological appearances of primary and metastatic tumour were similar, an essential criterion for the diagnosis of metastatic basal cell carcinoma (Lattes and Kessler, 1951). It was also evident that the lymph nodes had not become involved by local spread. The tumour in the lymph nodes was largely in a fibroblastic stroma but malignant cells were also present in subcapsular lymph sinuses, the first site where metastases are commonly seen.

Though unusual in the vulva, basal cell carcinoma is the most common skin cancer (Hughes, 1973; Wermuth and Fajardo, 1970) yet metastases are rare and there are only just over 100 such documented cases to date (Safai and Good, 1977). The absence of metastases in a series of 11 patients with basal cell carcinoma of the vulva (Cruz-Jimenez and Abell, 1975) and in reviews of 65 and 113 patients by Palladino *et al* (1969) and Breen *et al* (1975), respectively, supports the views that basal cell carcinoma of the vulva does not metastasize. However, Jimenez *et al* (1975) described lymph node involvement secondary to a left labial tumour and there are other similar reports (Temesvary, 1926; Eichenberg, 1934; Grimmer, 1968) but

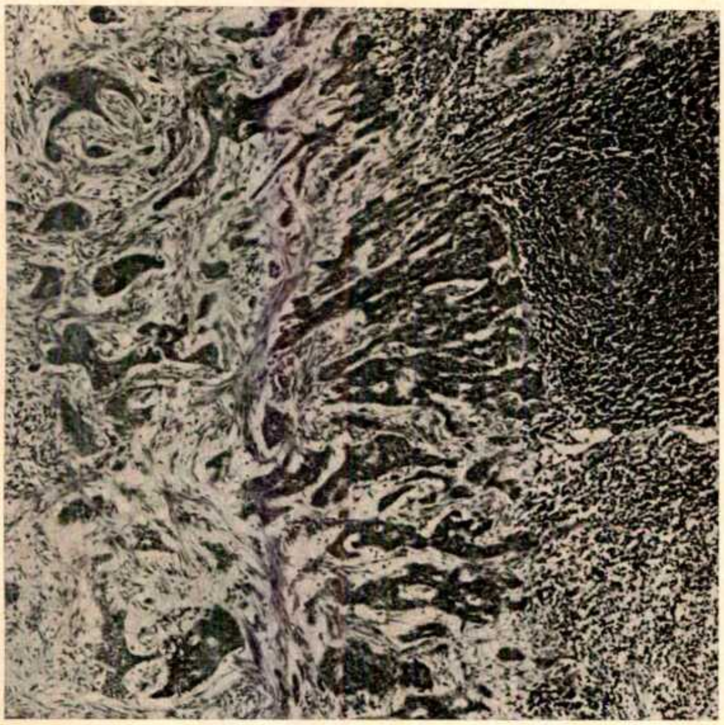

FIG. 2

Lymph node containing aggregates of hyperchromatic tumour cells in a dense fibroblastic stroma (haematoxylin and eosin ×13).

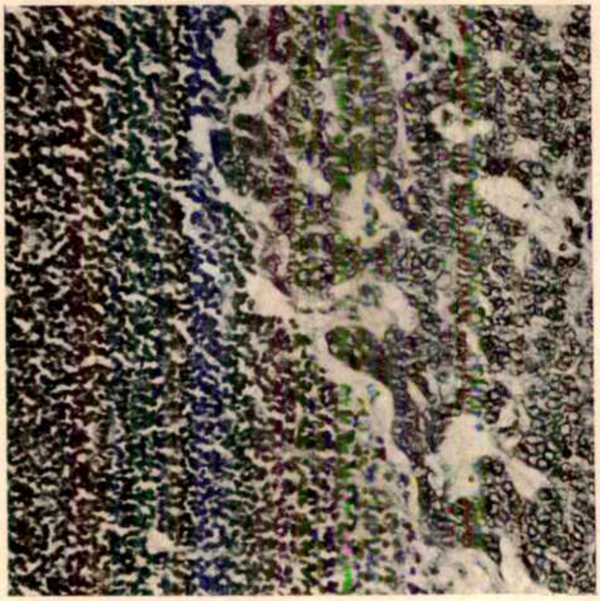

FIG. 3

Lymph node containing an aggregate of tumour cells with large ovoid nuclei and scanty cytoplasm (haematoxylin and eosin × 33).

photomicrographs do not show conclusively that the tumour in the lymph nodes is basal cell carcinoma.

The prognosis of metastatic basal cell carcinoma of the vulva is probably similar to that of basal cell carcinoma at other skin sites. Costanza *et al* (1974) found a mean survival time of ten months if there were distant metastases while Conway and Hugo (1965) recorded survivals of ten years or more if only regional lymph nodes were involved.

The treatments advocated for basal cell carcinoma of the vulva are wide local excision (Breen *et al*, 1975; Cruz-Jimenez and Abell, 1975) or irradiation. With palpable lymph node metastases, radical vulvectomy would seem advisable.

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BOOK REVIEWS

Recent Advances in Ultrasound Diagnosis. Edited by ASIM KURJAK. Pp 331, illustrated. Excerpta Medica, Amsterdam, 1978. Price: USA $52.25 (Dfl. 120.00).

THIS book, edited by Dr Kurjak, is in effect the proceedings of an International Symposium on Recent Advances in Ultrasound Diagnosis organised by the editor and held in Dubrovnik, Yugoslavia in October 1977. While the contributors, Dr Kurjak and the publishers are to be commended for the rapidity with which it has been produced, the book is no more nor no less than a collection of individual papers with no real thread of continuity. The contributors include many of the leading world authorities in this rapidly expanding field and in general their papers are of a good standard. However, there are a number which might usefully have been excluded, or at least have been substantially edited. The scope of the book spans most areas of ultrasound diagnosis from breast and ophthalmic scanning to examination of the liver and pancreas, with the obstetric component being one of the better sections. Those papers on intrauterine fetal 'breathing' movements deserve particular mention since they provide a good balance of the 'state of the art' and current research in this exciting area of investigation. A section on New Technical Developments makes interesting reading for the enthusiast with papers on real-time scanners, automated scanning techniques and Doppler devices, among others. It is a book which would be a useful addition to the library of an ultrasound department but is unlikely to attract the interest of obstetricians in general.

H. P. Robinson

Genitourinary Problems in Women. By J. ROBERTSON. Pp 149, illustrated. Charles C. Thomas, Illinois, USA, 1978. Price: $16.75.

DR JACK ROBERTSON is well known in the United States for his efforts in promoting gynaecological urology. He is a gynaecologist trained in the famous Kelly tradition at John Hopkins and has distilled 15 years experience of office practice in this speciality into the book. All too often we remain unaware of the lessons of history which have lead to present day practice and we are succinctly reminded of these in the second chapter. He emphasizes the importance of the urethra as the causation of many urological symptoms in the female and has produced a number of refinements to the gynaecologists' armentarium viz. Robertson's urethroscope and his suprapubic catheter introducer. The latter is particularly useful for ease of insertion of a 15 Fr. catheter. He emphasizes the importance of endoscopy in the investigation of urological symptoms and the value of carbon dioxide cystometry and urethral pressure profile measurements in their evaluation. The use of carbon dioxide rather than water in this context is still controversial here and indicates the need for future objective comparative observations. The anatomy and physiology of micturition are succinctly summarized and there are useful chapters on bladder drainage and urinary tract infections. This book is interestingly written and well illustrated and could be read with benefit by those interested in this area of specialization.

S. L. Stanton

Risks, Benefits, and Controversies in Fertility Control. Edited by J. J. SCIARRA, GERALD I. ZATUCHNI, and J. J. SPEIDEL. Pp xxi+601, illustrated. Harper & Row, Hagerstown, 1978. Price: $27.50.

IN March 1977 a group of workers, several with an international reputation, met in Arlington, Virginia, to discuss various aspects of fertility control. The papers presented are collected under nine headings: general aspects of fertility control, sterilization, steroid contraception—in relation to thromboembolism, neoplasia, metabolic and endocrine effects—pregnancy termination, intrauterine contraception, rhythm and barrier methods and the need for improved contraceptive techniques. Some papers review a whole field but others present new data on some narrow aspect of a problem. Some of the later presentations are based on few data and were hardly worth publishing. In contrast, the reviews are well referenced and were up to date in 1977. Malcolm Potts, Joseph Speidel and Elton Kessel examine the relative risks of various means of fertility control when used in less developed countries. They show that in least well-developed countries, oral contraception is safer at any age than unlimited childbearing while this is not true after the age of 35 in developed countries. Their analysis suggests that the least dangerous method of contraception in any country is barrier contraception backed up by legal abortion. The section on mid-trimester abortion provides a useful assessment of the risks of the various techniques and suggests that saline may be a little safer than prostaglandins and that dilation and evacuation, after suitable preparation of the cervix with laminaria tents, may be the least complicated method of all (in skilled hands). The long term risks of abortion are discussed but the conclusion is reached that none of the research done so far is entirely satisfactory but that long term sequelae are relatively uncommon. The section on intrauterine devices contains two useful discussions on the management of the intraperitoneal intrauterine device by Jack Lippes and by Richard Soderstrom. The latter considers that all such devices should be removed but the former suggests that inert linear devices should be left when they are in symptom-free women. I found that this book usefully up-dated my knowledge and provided easy access to references describing recent research. It will loose academic value quite rapidly but at present deserves a place in postgraduate libraries.

D. B. Paintin

Gynecology. By L. PARSONS and S. C. SOMMERS. Pp xxxix + 1660, illustrated. Holt-Saunders, Eastbourne, 1978. Second edition. Price: £35.00.

A REMARKABLE feature of this book of cradle-to-grave gynaecology (weighing 3·65 kg and measuring 27 × 19 × 7·5 cm) is that most of the subheadings are in the form of questions, academic as well as practical. This makes it ideal for anyone consulting it on a specific point, e.g. How does light affect the pineal gland? or Why do patients have premenstrual tension? This approach and the very size of the book will make it a reference book rather than a *vade mecum*. The authors, Dr Parsons a gynaecologist from Boston and Dr Sommers a pathologist who shares his work between New York and Los Angeles have provided a clear exposition of their views, with copious illustrations and with references up to 1977. The cover of topics is comprehensive, including the breasts, but there are occasional unexpected omissions. Prolactin does not figure in the index and bromocriptine is mentioned only in relation to galactorrhoea, perhaps because not yet released for use in the USA. Doyle's operation but not presacral neurectomy is quoted for secondary dysmenorrhoea, a symptom for which, interestingly, food allergies are said to be possible causes. But this volume raises its own questions. What is the place today for a book of over 1600 pages, especially when there is a 16 years' gap between editions? Would it not be preferable to have the work published in detachable sections, one or more of which could be replaced as required from time to time by relevant advances in knowledge and changes in practice?

S. Bender

Obstetric Emergencies, Second Edition. By D. CAVANAGH, R. WOODS and C. F. O'CONNOR. Pp 437 with illustrations. Harper & Row, Hagerstown (Maryland), 1978.

THE three authors of this book, two of them expatriates of our own National Health Service, are all obviously experienced and competent clinicians who have felt the need for a practical and easily read guide to the common obstetric emergencies which every practising obstetrician is likely to encounter during his clinical career. They take us clearly through antenatal, intrapartum and postpartum emergencies and have sensibly included the anaesthetic and fetal problems as well. The layout of each chapter is excellent with clearly defined subheadings allowing each emergency to be followed logically through its prevention, aetiology, diagnosis and treatment. The book is well illustrated and has good ultrasonic photographs but the excessive emphasis on this method of diagnosis will be misleading in this country where we will have to wait a long time before we see a competent ultrasonic department providing a 24 hour service in every hospital. Many of the chapters are excellent—particularly those on shock and clotting disorders but the quality is variable and I was disappointed with the chapter on elamptogenic toxaemia. Each chapter ends with an excellent up to date bibliography and recommendations for further reading. This is understandably slanted towards American publications but it is nice to see that our own journal is still read on the other side of the Atlantic. It is difficult to know for whom this book is intended. It is too detailed for the average undergraduate and chapters are insufficient for those studying for a higher qualification. I expect, however, the obstetric resident will find it a useful book for ready reference and certainly the actual practical procedures to be followed are well defined and safe.

A. Fraser

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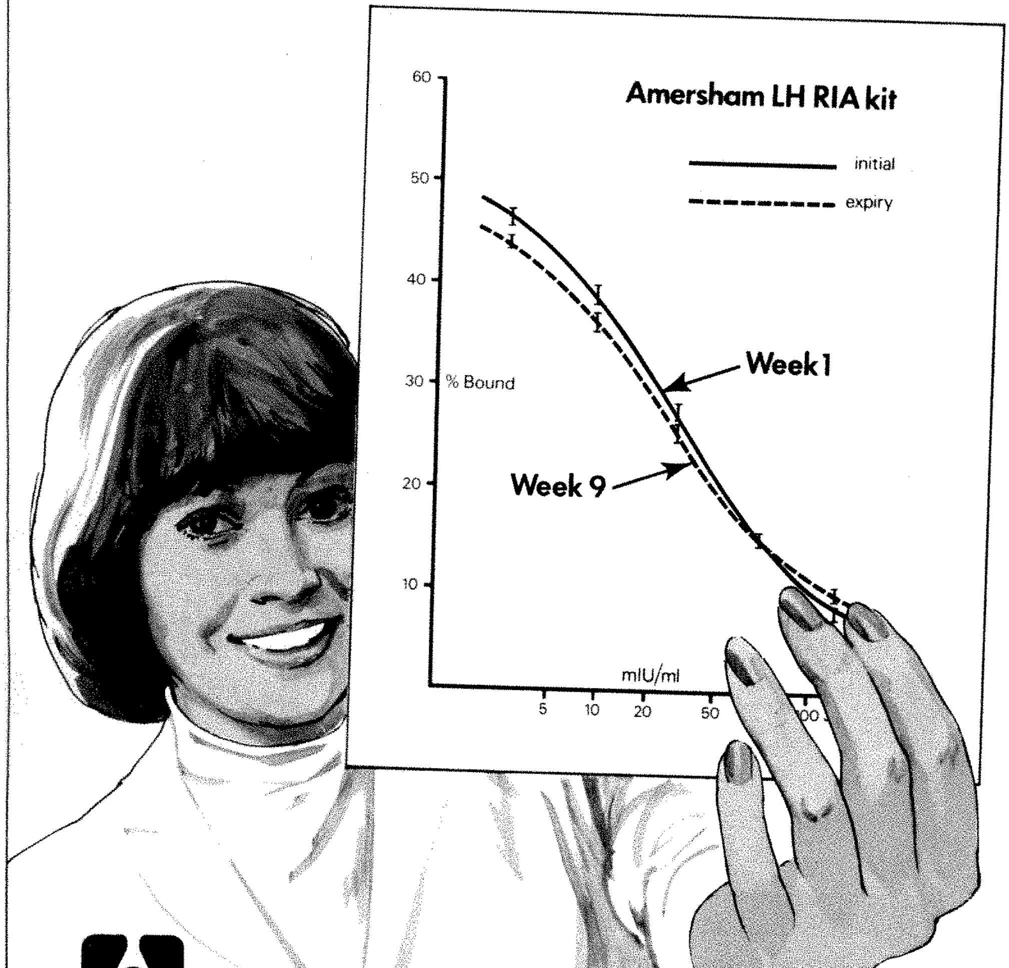

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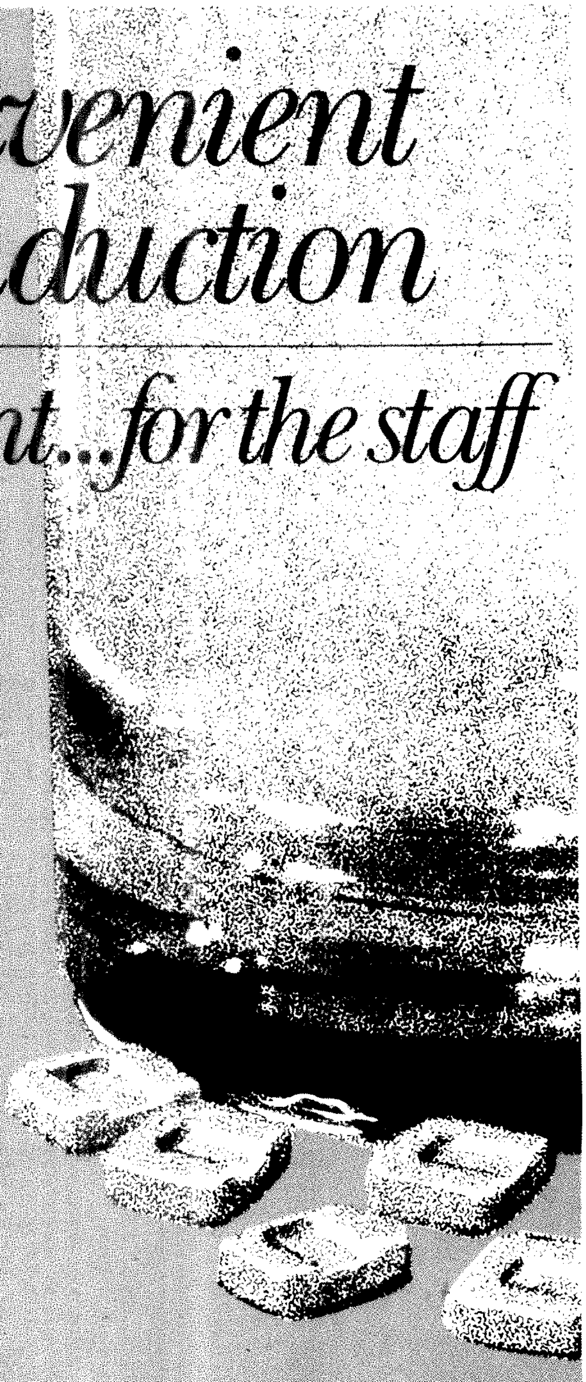

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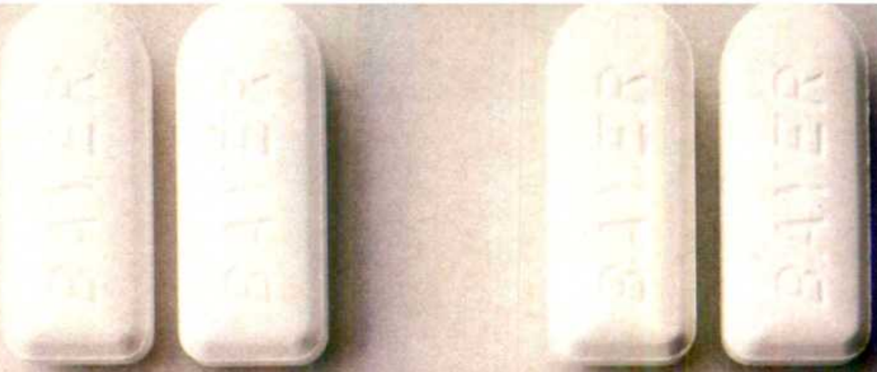

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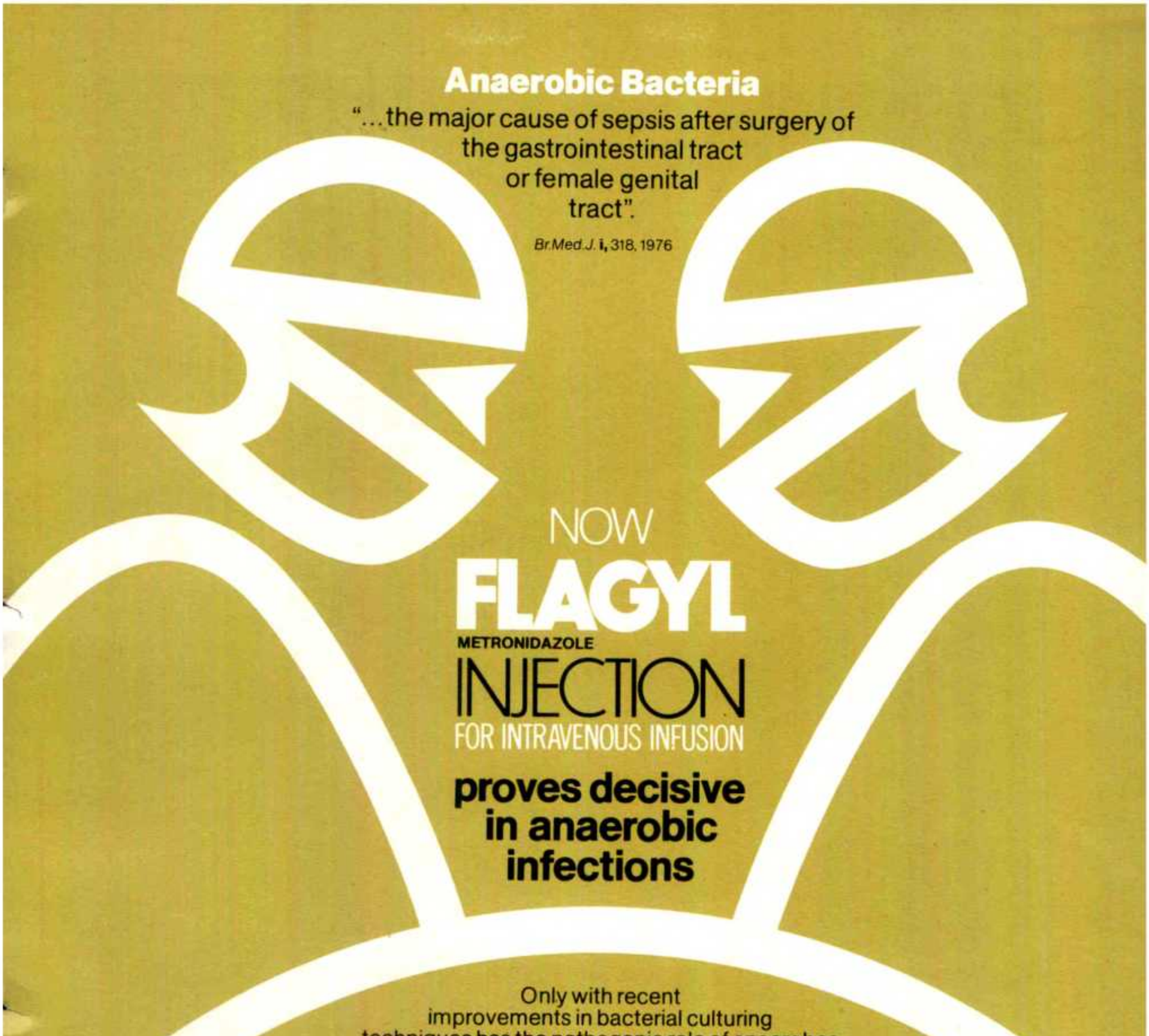

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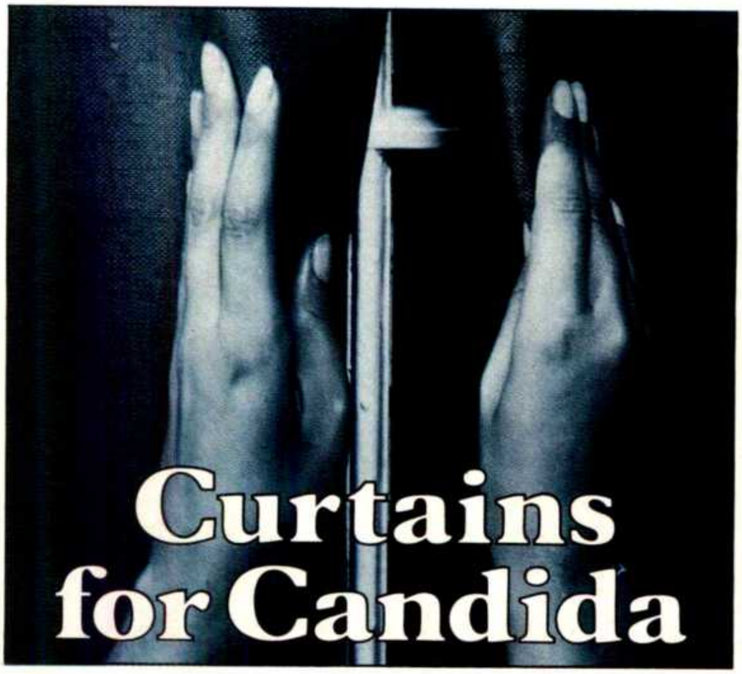


In just 3 nights

Ecostatin™

Econazole nitrate

Ecostatin™ eliminates candidal vaginitis in just 3 nights

- it is the most active fungicide against a wide range of pathogens[1]
- it combines potent fungistatic and fungicidal properties[2]

Ecostatin also ensures

- fast relief of symptoms
- no resistance[3]
- patient compliance

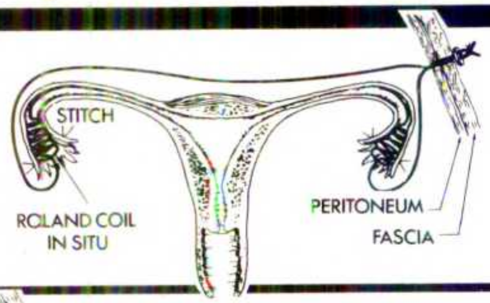
Ecostatin is also available as a cream for a wide range of dermatomycoses.

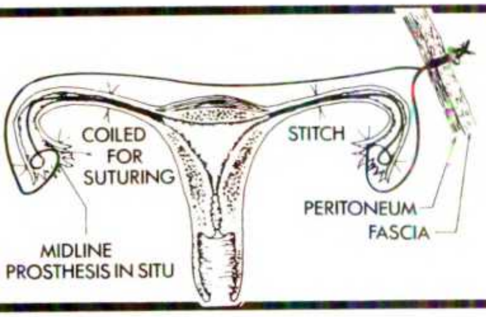
Prescribing Information
PRESENTATION
White, opaque oval pessaries each containing 150mg econazole nitrate in an hydrogenated vegetable oil base.
USES
Actions Econazole nitrate has a broad spectrum of antifungal activity. It is highly active against Candida albicans and other Candida species and is effective in controlling infections of the vagina and vulva caused by such organisms (thrush).
Indications Vulvovaginal candidosis.
DOSAGE AND ADMINISTRATION
One pessary to be inserted at bedtime for 3 consecutive nights. Administration should be continued even if menstruation occurs, and despite the disappearance of signs and symptoms of the infection.
The pessary should be inserted high into the vagina while the patient is supine. Although a three day course of therapy usually suffices it may be necessary to institute a second course of therapy.
CONTRA-INDICATIONS, WARNINGS, etc.
Contra-indications None known.
Precautions Ecostatin pessaries are effective in the candidal vaginitis associated with pregnancy. However, as with other agents, it should not be used during the first trimester of pregnancy unless the physician deems it's use essential for the welfare of the patient.
Side Effects Very occasionally patients may complain of discomfort; this is usually transitory and disappears with continued treatment. Rarely is it necessary to discontinue econazole pessary treatment.
PHARMACEUTICAL PRECAUTIONS
Storage Store at room temperature.
LEGAL CATEGORY POM
PACKAGE QUANTITIES Packs of three pessaries with applicator.
Price – 3 pessaries with applicator £3.60
Product Licence Number 0085/0013.
References
1. Avram, A. et Grupper, Ch. Bulletin de la Société Française de Mycologie Médicale 1975 Tome IV (2).
2. Preusser, H.J. et al. Research Report 1974. On file Cilag-Chemie.
3. Schweisfurth, R. Research Report 1974. On file Cilag-Chemie.

Ecostatin is a registered trade mark for econazole nitrate and FAIR Laboratories is the registered user.
Further information is available from:
FAIR Laboratories,
179 Heath Road, Twickenham TW1 4BJ

kli tuboplasty surgical prosthesis

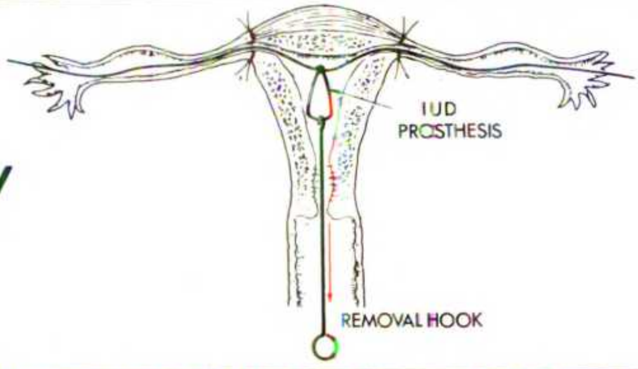
Stent for Fimbrial Tuboplasty





Stent for Midline Tuboplasty

Stent for Cornual Tuboplasty



Three disposable prostheses designed to maintain tubal patency following fimbrial, midline or cornual repair.

X-ray opaque. Simple technique for removal.

Write for more details of the prostheses, and information on our tubal surgical instruments.

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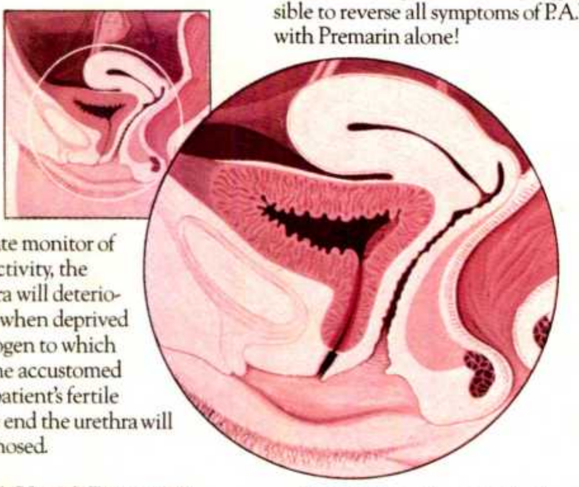
The Diagnosis and Treatment of P.A.U.

Post-menopausal Atrophic Urethritis

Older women presenting with frequency, urgency and painful micturition often describe their condition as 'cystitis.' There can hardly be a woman in existence who has not suffered the transient discomfort of a 'simple cystitis' at some time in her early life, but as she reaches middle age, although the symptoms may appear to her as the same, the cause, particularly in the absence of infection, may be atrophic changes in the distal urethra responding to hormonal disparities of the menopause.[1]

Untreated, these atrophic changes can be devastating and demoralising in their effect.[2]



A delicate monitor of oestrogen activity, the distal urethra will deteriorate rapidly when deprived of the oestrogen to which it has become accustomed during the patient's fertile years. In the end the urethra will become stenosed.

Treating P.A.U. with Premarin*

Treatment with Premarin 0.625mg tablets can effect a rapid and complete elimination of symptoms.[3]

1. Oestrogen deprivation can be confirmed by vaginal cytology or assumed in the presence of vaginal atrophy or dryness, or other symptoms of oestrogen lack.

2. Low-dose Premarin (0.625mg) administered cyclically – 21 days on, 7 days off – for 3 months. Urethral epithelium is remarkably sensitive to oestrogens and so will respond to this low dose.

3. Frank urinary tract infection should of course be treated with appropriate antibiotics or urinary anti-infectives.

4. If treatment is instigated in the early stages of this disorder – for instance within the first six/twelve months of symptoms – it may be possible to reverse all symptoms of P.A.U. with Premarin alone!

However in patients with a longer history of frequency and urgency, if oestrogen therapy does not effect a rapid and complete elimination of symptoms then the possibility of a secondary urethral stenosis should be considered.

References:

1. Willington, F. L. The Practitioner. 220, 739, 1978.
2. Smith, P. J. B., Ayerst Medical Library Vol. 2, No 2, 1978.
3. Smith, P. J. B., The Practitioner, 218, 97, 1977.

PREMARIN

(conjugated oestrogens)

Premarin tablets

Uses

Menopausal and post-menopausal oestrogen replacement therapy and allied disorders such as post-menopausal osteoporosis, atrophic vaginitis and post-menopausal atrophic urethritis.

Dosage and Administration

Menopausal and post-menopausal oestrogen deficiency: Premarin 0.625–1.25mg daily is the usual starting dose. Cyclic administration is recommended (three weeks' dosage followed by one week's rest). For maintenance, the lowest effective dose should be used. It is recommended that a physical examination, including endometrial assessment, whenever possible be performed at the time of the initial examination, and the endometrium assessed regularly thereafter at 6–12 month intervals. Break through bleeding is an indication for further investigation.

Contra-Indications: Uterine myomatosis. Endometriosis. Cancer of the breast and uterus. Active hepatic, renal and severe cardiac disease. Undiagnosed genital bleeding. Diabetes. Thyrotoxicosis. Pregnancy. Personal history of thrombosis.

Precautions

In the female – prolonged use of unopposed oestrogen may increase the risk of endometrial carcinoma.

Side Effects

Premarin is unlikely to cause gastro-intestinal symptoms, headaches, breast tenderness, salt and water retention which can occur with other forms of oestrogen therapy.

Product licence numbers

Premarin Tablets 0.625mg. PL 0607/5000.
21 tablet pack £0.80.
Premarin Tablets 1.25mg. PL 0607/5001.
21 tablet pack £1.30.
Premarin Tablets 2.5mg. PL0607/0002.
30 tablets £2.40.

Ayerst International

Full prescribing information:
Ayerst Laboratories Limited,
Invincible Road, Farnborough, Hants GU14 7QH.
Telephone: Farnborough (Hants) (STD 0252) 511981.

*Premarin is a Registered Trade Mark.

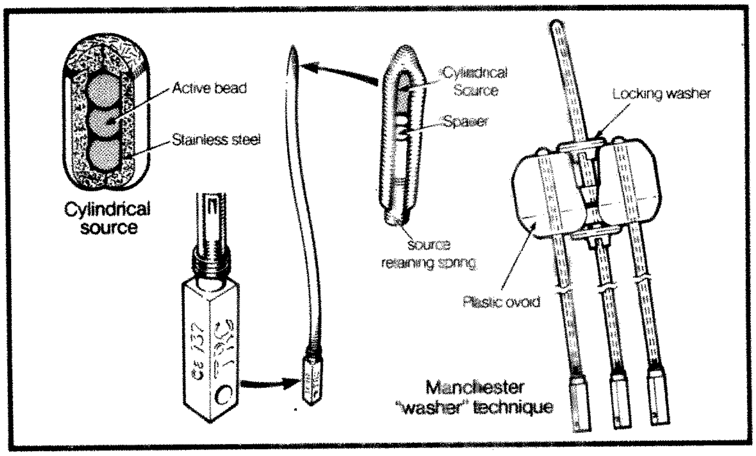
Our Afterloading Systems put brachytherapy right on target

With the addition of our new caesium-137 gynaecological afterloading system, we now supply a comprehensive range of products for intracavitary and interstitial radiotherapy.

Intracavitary radiotherapy

Our caesium-137 "Manchester" afterloading system consists of X-Ray opaque PVC applicators which allow the "washer," "spacer" or "tandem" techniques to be used. The guide tubes are flexible and have a diameter of only 6.3 mm overall. Complete kits are supplied sterilized in packaging designed for maximum convenience in use.

The sources contain glass beads in welded stainless steel capsules assembled into flexible source trains for which special shielded containers are supplied. Iso-dose curves are available for each source train. Individual sources and source trains are also supplied for most of the currently available remote afterloading systems.



Interstitial radiotherapy

Our iridium-192 flexible wire afterloading systems include complete sets of applicators and accessories for the Pierquin/Paine technique. All accessories for these systems are pre-packed ready for use and sterilized where necessary. Platinum clad iridium-192 wire in 500 mm lengths is readily available in activities up to approximately 0.3 mCi/mm providing exposure rates of up to 0.16 mR per hour at 1 metre.

Iridium-192 "hairpins" and single pins: Thicker platinum coated iridium-192 wire is supplied pre-shaped as "hairpins" and single pins for use with slotted guide needles which are made in stainless steel to the designs of Pierquin and Paine.

**The Radiochemical Centre
Amersham**

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In the Americas: Amersham Corporation, Illinois 60005. Telephone: 312-593-6300
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1885

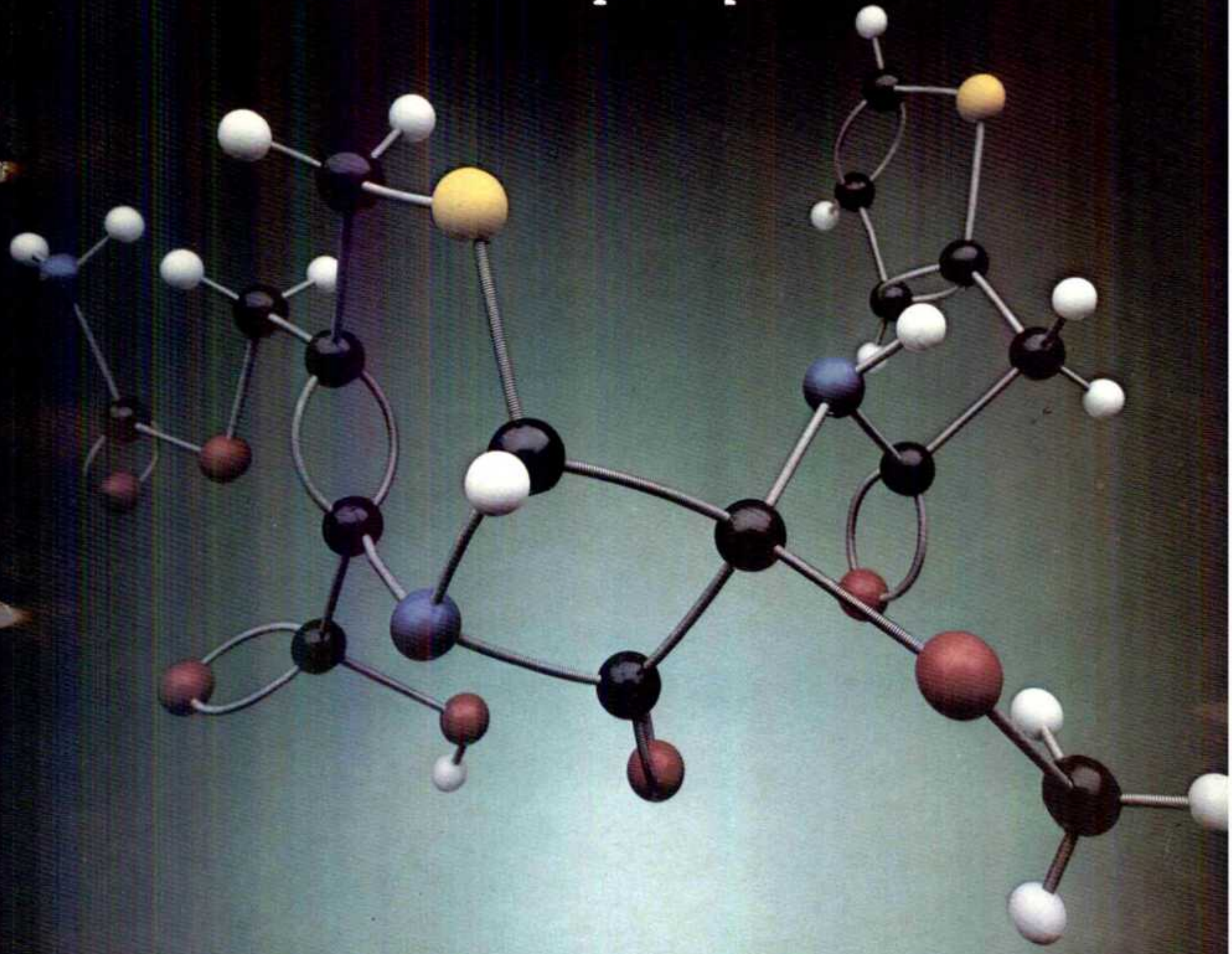
MSD announces
the discovery of

Mefoxin®

Cefoxitin sodium, MSD

INJECTION

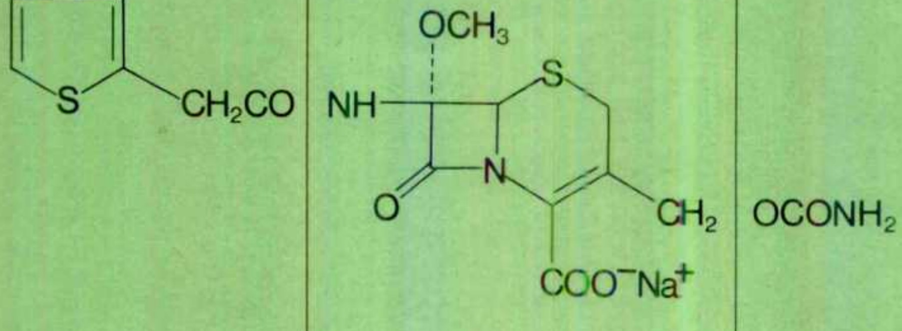
First of the cephamycins



A new class of antibiotics

'Mefoxin' is derived from cephamycin C, a naturally occurring antibiotic substance produced by *Streptomyces lactamdurans*. This naturally occurring antibiotic has been modified in the MSD Research Laboratories to enhance its therapeutic usefulness.

'Mefoxin' has a unique molecular structure offering outstanding clinical benefits and a specific clinical profile.



Thienylacetyl side-chain:

enhances activity against Gram-positive pathogens

Cephamycin nucleus:

provides bactericidal activity against a wide range of pathogens, Gram-negative in particular. 7α-methoxy group protects against β-lactamase degradation

Carbamoyloxy side-chain:

protects antibiotic from being metabolised

▼Prescribing information

Indications

'Mefoxin' is a broad-spectrum bactericidal antibiotic indicated for the treatment of infections caused by susceptible strains of Gram-positive and Gram-negative pathogens both aerobic and anaerobic.

'Mefoxin' has been clinically effective not only in infections due to antibiotic-sensitive organisms, but also in infections due to organisms resistant to one or more of the following antibacterial agents: penicillin, ampicillin, carbenicillin, tetracyclines, erythromycin, chloramphenicol, cephalosporins, kanamycin, gentamicin, tobramycin, and sulphamethoxazole-trimethoprim.

Many Gram-negative pathogens are resistant to penicillins and cephalosporins through the action of the β-lactamases which are produced by these pathogens. 'Mefoxin' is remarkably stable in the presence of these bacterial β-lactamases, both penicillinases and cephalosporinases. Hence, the clinical efficacy of 'Mefoxin' extends to many infections caused by such pathogens.

'Mefoxin' is indicated for the treatment of the following infections caused by sensitive bacteria:
Peritonitis and other intra-abdominal and intrapelvic infections; female genital tract infections; septicaemia; urinary tract infections; respiratory tract infections; bone and joint infections; and skin and soft-tissue infections.

'Mefoxin' is indicated for adjunctive therapy in the surgical treatment of infections, including abscesses, infection complicating hollow visceral perforations, cutaneous infections, and infections of serous surfaces, whether caused by aerobes, mixed aerobes and anaerobes, or anaerobes.

Microbiology

'Mefoxin' is active *in vitro* against:

Aerobic bacteria

Gram-positive cocci including: Staphylococci: (including coagulase-positive, coagulase-negative, and penicillinase-producing strains); Group A beta-haemolytic streptococci *(Streptococcus pyogenes)*; Group B beta-haemolytic streptococci *(Streptococcus agalactiae)*; *Streptococcus pneumoniae (Diplococcus pneumoniae)*; other streptococci (except group D streptococci including faecalis). *Gram-negative cocci including: Neisseria gonorrhoeae* (including penicillinase-producing strains); *Neisseria meningitidis*; *Gram-negative rods (facultative anaerobes) including: Escherichia coli*; *Klebsiella pneumoniae*; Klebsiella spp.; *Proteus mirabilis*; Proteus (indole-positive): *Proteus vulgaris*; *Proteus rettgeri*; *Proteus morgani*; *Haemophilus influenzae*; *Serratia marcescens*; Providencia spp.; Salmonella and Shigella spp.

Anaerobic bacteria

Gram-positive cocci including: Peptococcus spp.; Peptostreptococcus spp.; Micro-aerophilic streptococcus. *Gram-positive rods including: Clostridium perfringens*; Clostridium spp.; Eubacterium spp.; *Propionibacterium acnes*. *Gram-negative cocci including:* Veillonella spp. *Gram-negative rods including: Bacteroides fragilis*; *Bacteroides melaninogenicus*; Bacteroides spp. (including both penicillin-susceptible and penicillin-resistant strains); Fusobacterium spp.

'Mefoxin' is active against some strains of the following bacteria: *Acinetobacter* calcoaceticus var anitratum *(Herellea vaginicola)*; *Acinetobacter* calcoaceticus var *lwoffi (Mima polymorpha)*; *Alcaligenes faecalis*; Citrobacter spp. and Flavo-bacterium spp.; Enterobacter spp.

'Mefoxin' is not active against Pseudomonas, most strains of enterococci and many strains of *Enterobacter cloacae*.

Dosage and administration

'Mefoxin' may be administered intravenously or intramuscularly. (See reconstitution directions for each route below.) Dosage and route of administration should be determined by severity of infection, susceptibility of the causative organisms, and condition of the patient.

Therapy may be started while awaiting the results of susceptibility testing.

Dosage: The usual adult dosage is 1 g or 2 g of 'Mefoxin' every eight hours. (See chart.)

In adults with renal insufficiency, an initial loading dose of 1 g or 2 g

Usual adult dosage

Type of infection	Dose	Frequency	Total daily dosage
Uncomplicated	1 g	Every 8 hours (occasionally every 6 hours)	3 g (4 g)
Moderately severe or severe	2 g	Every 8 hours (occasionally every 6 hours)	6 g (8 g)
Infections generally needing antibiotics in higher dosage	3 g (2 g)	Every 6 hours (Every 4 hours)	12 g

Maintenance dosage of 'Mefoxin' in adults with reduced renal function

Renal function	Creatinine clearance (ml/min)	Dose	Frequency
Mild impairment	50-30	1-2 g	Every 8-12 hours
Moderate impairment	29-10	1-2 g	Every 12-24 hours
Severe impairment	9-5	0·5-1 g	Every 12-24 hours
Essentially no function	<5	0·5-1 g	Every 24-48 hours

In the patients undergoing haemodialysis, the loading dose of 1 to 2 g should be given after each haemodialysis, and the maintenance dose should be given as indicated in the table above.

Intravenous administration: reconstitute 'Mefoxin' with Water for Injection BP: 1 g is soluble in 2 ml. Although 'Mefoxin' is very soluble, for intravenous use it is preferable to add 10 ml of Water for Injection BP to the 1 g vial or the 2 g vial. Shake to dissolve and then withdraw entire contents of vial into syringe.

Outstanding clinical benefits

Wide-ranging activity against Gram-negative and Gram-positive pathogens, anaerobes as well as aerobes.

Unusually high resistance to β-lactamase degradation.

Highly active against *Bacteroides fragilis* and strains of *E coli,* Serratia, Klebsiella spp. and Proteus spp. (including indole positive strains) resistant to other antibacterial agents.

Offers high levels of active antibiotic in the body.

A record of relative safety comparable to the penicillins.

Prime indications include:

- Peritonitis, and other intra-abdominal and intrapelvic infections.
- Female genital tract infections
- Septicaemia
- Skin and soft tissue infections
- Bone and joint infections
- Urinary tract infections
- Respiratory tract infections (e.g. inhalation pneumonia)

Specific clinical profile

'Mefoxin' is exceptionally valuable wherever Gram-negative pathogens and anaerobes are involved in mixed infections. When mixed infections are suspected, therapy may be started while awaiting the results of susceptibility testing.

Mefoxin®

Cefoxitin sodium, MSD

INJECTION

First of the cephamycins

the vein over a period of 3 to 5 minutes or may be given through the tubing when the patient is receiving parenteral solutions.

An intermittent intravenous infusion of 'Mefoxin' may be employed when large amounts of fluid are to be given. However, during infusion of the solution containing 'Mefoxin', it may be advisable temporarily to discontinue administration of any other infusion solution at the same site (by using an appropriate IV infusion set).

A solution of 'Mefoxin' may also be given by continuous intravenous infusion (see below for compatibility and stability).

Intramuscular administration ONLY
Reconstitute 'Mefoxin' 1 g with 2 ml of Water for Injection BP, or 0.5% or 1% lignocaine hydrochloride (without adrenaline) solution. 'Mefoxin' is given by deep injection into a large muscle mass. Avoid injection into a blood vessel.

Compatibility and stability
A solution of 'Mefoxin' in Water for Injection BP may be added to the following solutions: 0.9% Sodium Chloride Injection BP, 5% or 10% Dextrose Injection BP, Dextrose and Sodium Chloride Injection (5%/0.9%, 5%/0.45%, or 4.3%/0.18%), Lactated Ringer's Injection USP, 5% Dextrose in Lactated Ringer's Injection, 5% or 10% invert sugar in water, 10% invert sugar in saline solution, 4.2% Sodium Bicarbonate Injection BP, M/6 Sodium Lactate Injection BP.

'Mefoxin', as constituted to 1 g/10 ml with Water for Injection BP, Water for Injection BP preserved with parabens or benzyl alcohol, 0.9% Sodium Chloride Injection BP, or 5% Dextrose Injection BP, maintains satisfactory potency for 24 hours at room temperature, for 96 hours under refrigeration, and for 13 weeks in the frozen state.

'Mefoxin' can be administered concomitantly with such antibiotics as gentamicin, tobramycin, carbenicillin or amikacin. When 'Mefoxin' is administered with another antibiotic, they should not be mixed in the same syringe.

Contra-indications
'Mefoxin' is contra-indicated in persons who have shown hypersensitivity to cefoxitin. In the absence of clinical experience,

Precautions
There is some clinical and laboratory evidence of partial cross-allergenicity between cephamycins and other β-lactam antibiotics, penicillins, and cephalosporins. Severe reactions (including anaphylaxis) have been reported with most β-lactam antibiotics.

Before therapy with 'Mefoxin', careful inquiry should be made concerning previous hypersensitivity reactions to β-lactam antibiotics. 'Mefoxin' should be given cautiously to penicillin-allergic patients.

Any patient who has demonstrated some form of allergy, particularly to drugs, should receive antibiotics cautiously. If an allergic reaction to 'Mefoxin' occurs, the drug should be discontinued.

The total daily dosage should be reduced when 'Mefoxin' is administered to patients with transient or persistent reduction of urinary output due to renal insufficiency (see 'Dosage and administration') because high and prolonged serum antibiotic concentrations can occur from usual doses.

Interference with laboratory tests
A false-positive reaction to glucose in the urine may occur with reducing substances but not with the use of specific glucose oxidase methods.

Use in pregnancy
Use of the drug in women of childbearing potential requires that the anticipated benefits be weighed against possible hazards. Reproductive and teratogenic studies have been performed in mice and rats and have revealed no evidence of impaired fertility or harm to the foetus due to 'Mefoxin'. There are no controlled studies with 'Mefoxin' in pregnant women.

Nursing mothers
'Mefoxin' is excreted in human milk.

Infants and children
Pending the outcome of clinical trials in progress, the conditions for use of 'Mefoxin' in infants and children remain to be defined.

stopping treatment and usually have been mild and transient.

Local reactions
Thrombophlebitis has occurred with intravenous administration. Pain, induration and tenderness after intramuscular injections have been reported.

Allergic
Maculopapular rash, urticaria, pruritus, eosinophilia, fever and other allergic reactions have been noted rarely.

Gastro-intestinal
Diarrhoea has been reported rarely.

Blood
Transient leucopenia and neutropenia have been reported. Some individuals, particularly those with azotaemia, may develop positive direct Coombs tests during therapy with 'Mefoxin'.

Liver function
Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase have been reported rarely.

Kidney
Elevations in serum creatinine and/or blood urea levels have been observed. The role of 'Mefoxin' in changes in renal function test is difficult to assess, since factors predisposing to pre-renal azotaemia or to impaired renal function usually have been present.

Presentation
In vials containing 1 g or 2 g of cefoxitin as the sodium salt. Each gram of cefoxitin contains approximately 51.5 mg (2.2 mEq) sodium.

Product licence numbers: 1 g, 0025/0130; 2 g, 0025/0131

Product authorisation numbers: 1 g, PA 35/42/1; 2 g, PA 35/42/2

Issued October 1978

Additional information is available to the medical profession on request.

Basic NHS cost is £4.47 for the 1 g vial, and £8.94 for the 2 g vial.

MSD

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COMPLOMENT Continus tablets will effectively ensure adequate supplementation of the pyridoxine from a single tablet, taken daily.

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Dosage and Administration
One tablet to be taken daily, or as directed by the Physician.

Price Per Day
3.5 pence

NAPP Napp Laboratories Limited,
Hill Farm Avenue,
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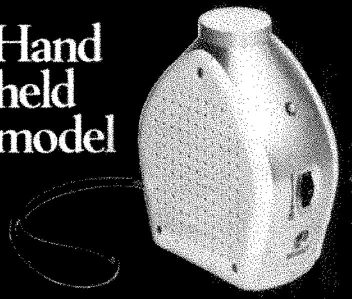
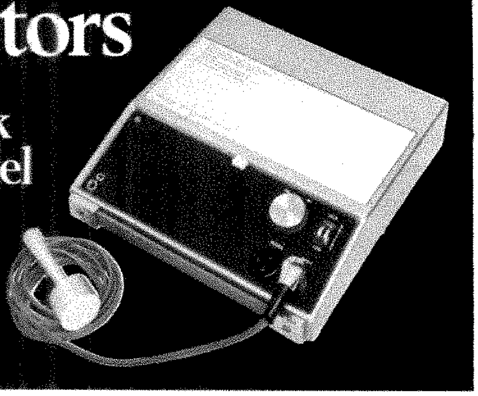
*Ref. Lancet (1973), 1,897

Fetal Pulse Detectors

Hand held model



Desk model



Downs Surgical present the latest portable units for detection of early fetal life. Both units operate using the ultrasound Doppler principle which enables the heartbeat to be detected as early as 10 weeks gestation. Internal speakers are fitted to both units and headphones are supplied for personal listening.

Downs Surgical

To Downs Surgical Limited,
Church Path, Mitcham, Surrey CR4 3UE, England.
Please send me details of Fetal Pulse Detectors

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Address ______________________

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Prescribing Information

Presentation
White egg-shaped pessaries each containing 150mg econazole nitrate and melting at 37°C.
Uses
Vaginitis due to Candida albicans and other yeasts.
Dosage and Administration
One pessary should be inserted high into the vagina each evening for three consecutive days.
Contra-indications, Warnings etc.
Contra-indications:
None known.
Side-effects:
None known.
Overdosage:
Not applicable.
Precautions:
Hypersensitivity has never been recorded, but if it should occur, administration of the product should be discontinued.
Gyno-Pevaryl* 150 vaginal pessaries are effective in the treatment of vaginal candidiasis associated with pregnancy but as with any new product, caution should be exercised when prescribing during the first trimester.
Pharmaceutical Precautions
Store in a cool place.
Legal Category
Prescription only (POM).
Package Quantities
Each pack contains 3 pessaries and a patient instruction leaflet.
The pessaries are individually sealed in a white plastic strip marked **Gyno-Pevaryl* 150** at regular intervals.
Further Information
Anogenital hygiene is important to help prevent re-infection.
PL0076/0058
Basic NHS Price – £3.60
Name and address of Licensee
Ortho Pharmaceutical Limited, P.O. Box 79,
Saunderton, High Wycombe,
Buckinghamshire
HP14 4HJ, England.
Further information and data sheet available on request.

Ortho

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NEW PRODUCT

The quick way to say Gyno-Pevaryl*

Vaginal Pessaries

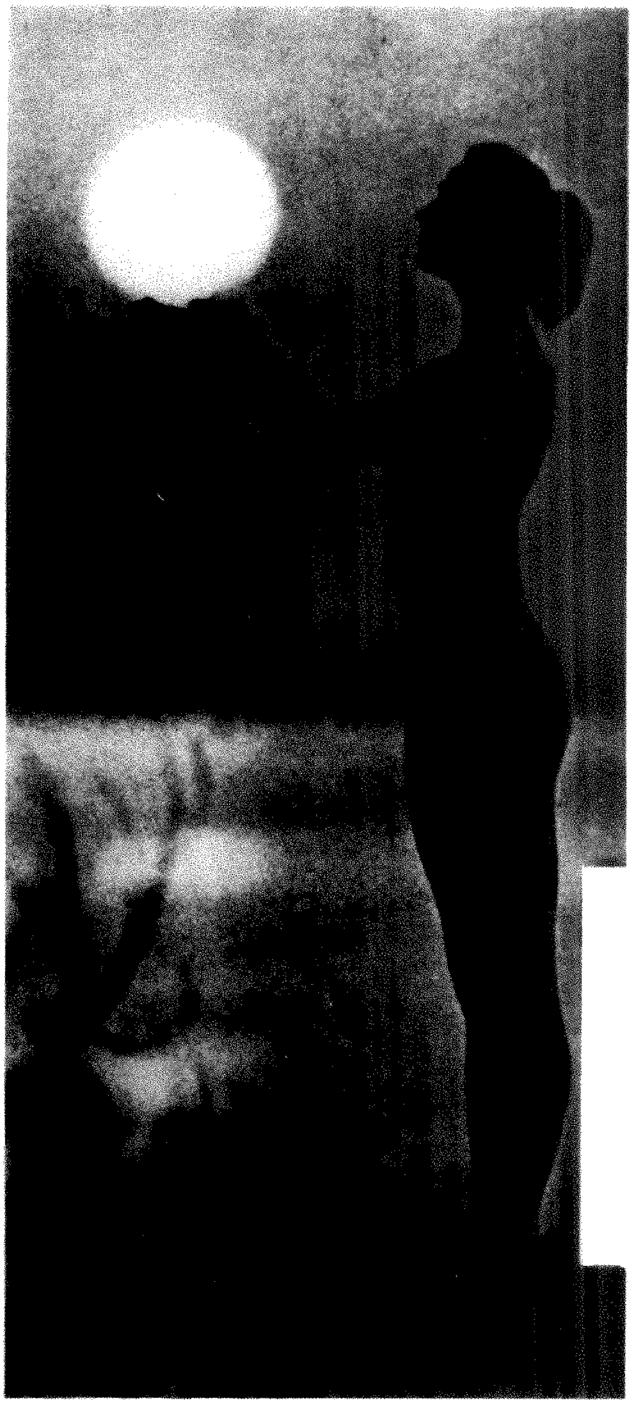
GP

The effective 3 day, 3 pessary treatment for vaginal candidiasis

Rapid symptomatic relief Burning pruritus and discharge promptly subside on therapy.
Effective in over 85% of cases – in only 3 days[1] Three days are all it takes to clear the condition in over 85% of cases.[1]
Better patient compliance Three rapidly dissolving soft-wax pessaries inserted one at night for three days, effectively minimise the possibility of recurrence through non-compliance by reducing the therapy to its simplest form ever. **Gyno-Pevaryl* 150** vaginal pessaries – easy, quick and effective in the treatment of vaginal candidiasis.

NEW
Gyno-Pevaryl*150
Vaginal Pessaries **Econazole Nitrate 150mg.**
new-generation antifungal
the quick way to treat vaginal candidiasis

* Trademark ©OPL 1978 Reference: 1. Balmer, J.A. (1976). Am J. Obstet.Gynae. 126, 4, 436-441.



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- in combining electroanalgesia in caesarean operations
- in combining endotracheal anaesthesia by nitrous oxide plus electroanalgesia in gynaecological operations
- in prenatal treatment as a means of prophylaxis against anomalies during labours
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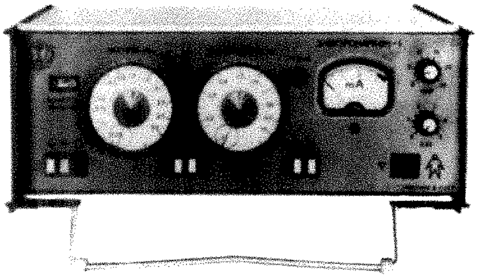

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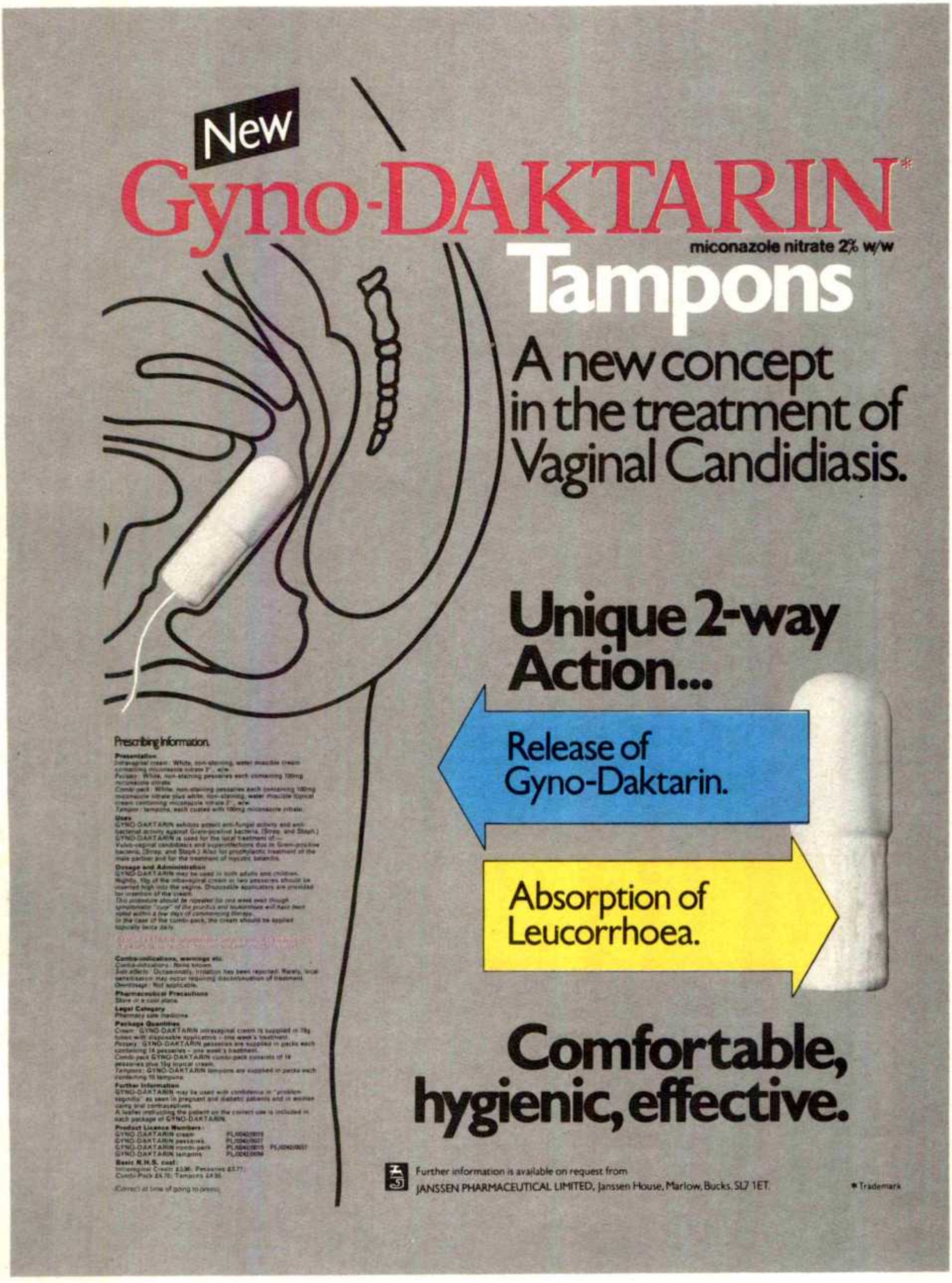

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[illegible]

Contra-indications, warnings etc.
Contra-indications: None known.
Side effects: Occasionally, irritation has been reported. Rarely, local sensitisation may occur requiring discontinuation of treatment.
Overdosage: Not applicable.

Pharmaceutical Precautions
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PRESENTATION

NEOPLATIN INJECTION: each vial contains 10 mg cisplatin (Cis-diamminedichloroplatinum).

USES

Cisplatin is indicated as palliative therapy to be employed in addition to other modalities, or in established combination therapy with other approved chemotherapeutic agents in the management of neoplastic diseases, especially:

Metastatic Testicular Tumours – in patients who have already received appropriate surgical and/or radiotherapeutic procedures. The combination of cisplatin, bleomycin and vinblastine has been reported to be highly effective. Other combinations have also been reported to be active.

Metastatic Ovarian Tumours – as secondary therapy in patients refractory to standard chemotherapy.

DOSAGE AND ADMINISTRATION

The recommended dose of cisplatin in adults and children as single-agent therapy is 50 to 75 mg/m^2 as a single i.v. dose every 3 to 4 weeks, or 15 to 20 mg/m^2 i.v. daily for 5 days every 3 to 4 weeks. Pre-treatment hydration with 1 to 2 litres of fluid infused for 8 to 12 hours prior to a cisplatin dose is recommended. Reconstitute vial contents with 10 ml of sterile water for Injection EP. The drug is then diluted in 2 litres of 5% dextrose in either 1/2 or 1/3 normal saline, (to which 37.5 g of mannitol may be added) and infused over a 6-8 hour period. Adequate hydration and urinary output must be maintained during the following 24 hours.

A repeat course of cisplatin should not be given until the serum creatinine is below 1.5 mg/100 ml (130 μmol/l) and/or the blood urea below 55 mg/100 ml (9 mmol/l). A repeat course should not be given until circulating blood elements are at an acceptable level (platelets 100,000/mm^3, WBC 4,000/mm^3). Subsequent doses of cisplatin should not be given until an audiometric analysis indicates that auditory acuity is within normal limits. When employed in combination with other antitumour drugs, the dose of cisplatin should be adjusted appropriately.

CONTRA-INDICATIONS, WARNINGS, ETC.

Cisplatin induces nephrotoxicity, myelosuppression and ototoxicity which may be additive to pre-existing dysfunctions. Consequently, the use of cisplatin in patients with hearing or renal impairment or depressed bone marrow function may be associated with increased toxicities. Such conditions represent relative contra-indications.

Cisplatin is contra-indicated in patients with a history of hypersensitivity to cisplatin or other platinum-containing compounds.

WARNINGS

Cisplatin produces cumulative nephrotoxicity. The serum creatinine, BUN and creatinine clearance should be measured prior to initiating therapy, and prior to each subsequent course.

Anaphylactic-like reactions to cisplatin have been reported. These reactions have occurred within minutes of administration to patients with prior exposure to cisplatin, and have been alleviated by administration of adrenaline, steroids and antihistamines.

Safe use in pregnancy has not been established. This product should not normally be administered to patients who are pregnant or to mothers who are breast feeding. Cisplatin is mutagenic in bacteria and produces chromosome aberrations in animal cells in tissue culture. Although carcinogenicity and teratogenicity have not been established, compounds with similar mechanisms of action and mutagenicity have been reported to be carcinogenic.

The influence of cisplatin on human reproduction has not been determined. Cisplatin therapy might have an antifertility effect.

Precautions

Cisplatin should be administered by individuals experienced in the use of antineoplastic therapy.

Since renal toxicity is cumulative, measurements of BUN, serum creatinine and creatinine clearance should be performed prior to initiating therapy and prior to each subsequent dose. At the recommended dosage, cisplatin should not be given more frequently than once every 3 to 4 weeks. (See Adverse Reactions).

Since ototoxicity of cisplatin is cumulative, audiometric testing should be performed prior to initiating therapy and prior to each subsequent dose of drug. (See Adverse Reactions).

Peripheral blood counts should be monitored weekly. Liver function should be monitored periodically. Neurological examination should also be performed regularly. (See Adverse Reactions).

ADVERSE REACTIONS

Nephrotoxicity

Dose-related and cumulative renal insufficiency is the major dose-limiting toxicity of cisplatin. Renal toxicity has been noted in 28 to 36% of patients treated with a single dose of 50 mg/m^2. It is first noted during the second week after a dose and is manifested by elevations in BUN and serum creatinine, serum uric acid and/or a decrease in creatinine clearance.

Renal toxicity, becomes more prolonged and severe with repeated courses of the drug. Renal function must return to normal before another dose of cisplatin can be given.

Renal function impairment has been associated with renal tubular damage. The administration of cisplatin using a 6-8 hour infusion with intravenous hydration and mannitol has been used to reduce nephrotoxicity. However, renal toxicity still can occur after utilisation of these procedures.

Ototoxicity

Ototoxicity has been observed in up to 31% of patients treated with a single dose of cisplatin 50 mg/m^2, and is manifested by tinnitus and/or hearing loss in the high frequency range (4,000 to 8,000 Hz).

Decreased ability to hear normal conversational tones may occur occasionally. Ototoxic effects may be more severe in children receiving cisplatin. Hearing loss can be unilateral or bilateral and tends to become more frequent and severe with repeated doses. It is unclear whether cisplatin-induced ototoxicity is reversible. Careful monitoring of audiometry should be performed prior to initiation of therapy and prior to subsequent doses of cisplatin.

Haematological

Myelosuppression occurs in 25 to 30% of patients treated with cisplatin. The nadirs in circulating platelets and leucocytes occur between days 18 to 23 (range 7.5 to 45) with most patients recovering by day 39 (range 13 to 62). Leucopenia and thrombocytopenia are more pronounced at higher doses (50 mg/m^2). Anaemia (decrease in haemoglobin level by 2 g/100 ml blood) occurs at approximately the same frequency and with the same timing as leucopenia and thrombocytopenia.

Gastrointestinal

Marked nausea and vomiting occur in almost all patients treated with cisplatin, and are occasionally so severe that the drug must be discontinued. Nausea and vomiting usually begin within one to four hours after treatment and last up to 24 hours. Various degrees of nausea and anorexia may persist for up to one week after treatment.

OTHER TOXICITIES

Hyperuricaemia

Hyperuricaemia has been reported to occur at approximately the same frequency as the increases in BUN and serum creatinine. It is more pronounced after doses greater than 50 mg/m^2, and peak levels of uric acid generally occur between 3 to 5 days after the dose. Allopurinol therapy for hyperuricaemia effectively reduces uric acid levels.

Neurotoxicity

Neurotoxicity, usually characterised by peripheral neuropathies, has occurred in some patients. Loss of taste and seizures have also been reported. Neuropathies resulting from cisplatin treatment may occur after prolonged therapy (4 to 7 months), however, neurological symptoms have been reported to occur after a single dose.

Cisplatin therapy should be discontinued when the symptoms are first observed. Preliminary evidence suggests peripheral neuropathy may be irreversible in some patients.

Anaphylactic-like Reactions

Anaphylactic-like reactions, possibly secondary to cisplatin therapy, have been occasionally reported in patients previously exposed to cisplatin. The reactions consist of facial oedema, wheezing, tachycardia and hypotension within a few minutes of drug administration. All reactions may be controlled by intravenous adrenaline, corticosteroids or antihistamines. Patients receiving cisplatin should be observed carefully for possible anaphylactic-like reactions and supportive equipment and medication should be available to treat such a complication.

Other toxicities reported to occur infrequently are cardiac abnormalities, anorexia and elevated SGOT.

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The reconstituted solution should be protected from light, kept at room temperature and is stable for 20 hours. Refrigeration will result in precipitation.

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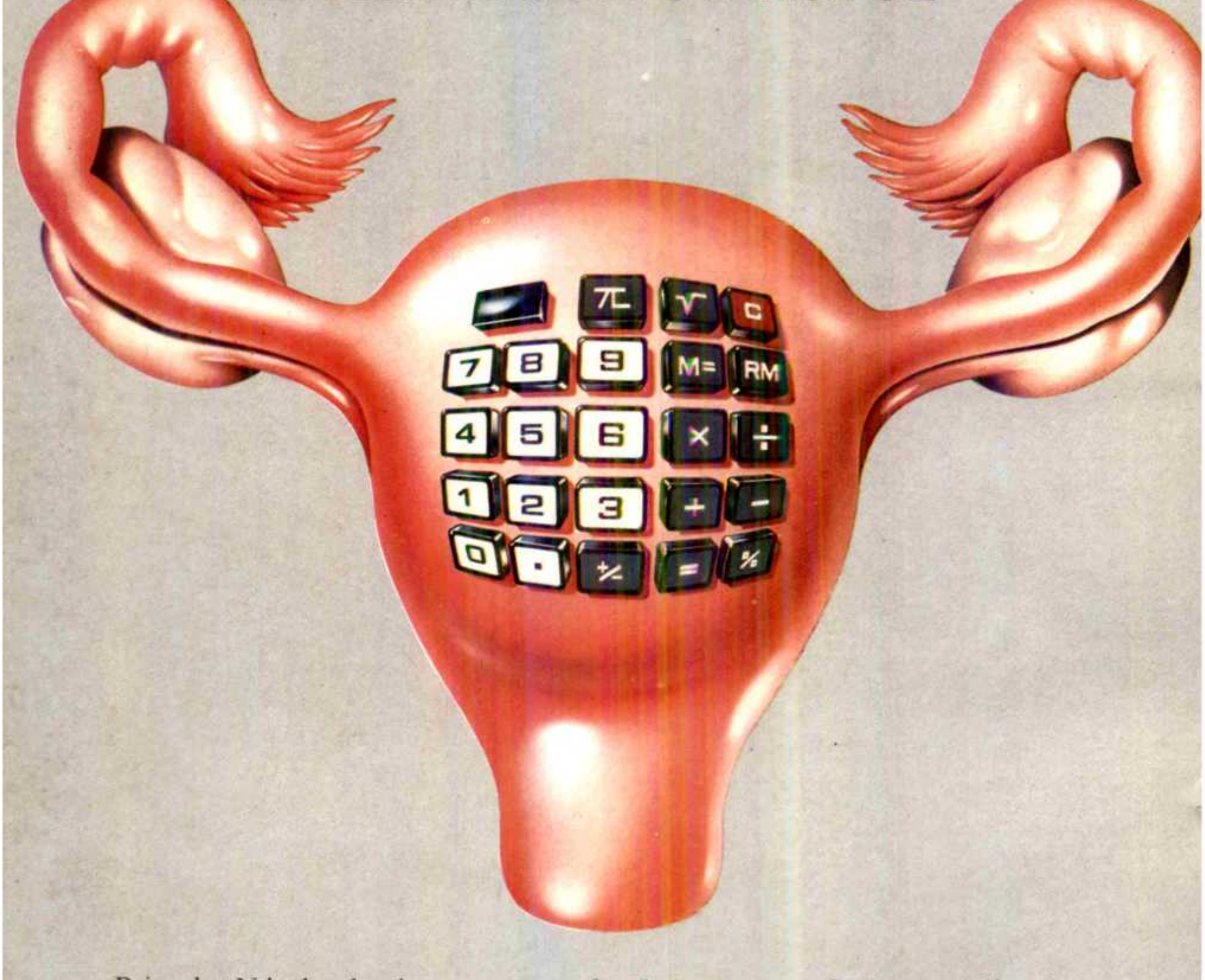

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British Journal of Obstetrics and Gynaecology

Vol 86 No. 5 NEW SERIES MAY 1979

CASE NOTE DESCRIPTIONS OF THE PLACENTA: ARE THEY WORTHWHILE?

BY

P. Penfold, *Research Officer*
Linda Drury, *Research Officer*
Leila Lewis, *Research Sister*
J. P. Royston, *Scientific Staff*

AND

F. E. Hytten, *Head of Division*

Divisions of Perinatal Medicine and Computing and Statistics
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Watford Road, Harrow, Middlesex HA1 3UJ

Summary

In 429 placentae, measurements were made of weight, diameter, shape, eccentricity of the cord and weight and length of the cord, and the results were compared with Apgar score of the infant at birth and its standardized birth weight. There was no evidence that cord eccentricity, placental shape or 'thickness', or the dimensions of the cord had any significant relation to the growth of the fetus or its condition at birth. The value of routine recording of crude measurements and qualitative assessments of the placenta in case notes is questioned.

It is traditional for midwives to record gross measurements of the placenta, usually weight and diameter, and the length of the umbilical cord. They also record observations about the site of implantation of the cord and such general remarks as that the placenta looks 'thin' or 'unhealthy' or that the cord is 'thin and stringy'.

The most consistently recorded of the measurements, placental weight, is exceptionally crude and includes widely varying amounts of membrane, umbilical cord and contained blood. Furthermore, the evidence is overwhelming that although the weight of the placenta, like any other fetal organ, is loosely related to the weight of the fetus, it is of negligible value as an indicator of functional adequacy (Fox, 1978).

This study set out to determine whether any of the other routinely recorded information about the placenta is any more valuable.

Methods

Placentae were collected for measurement from the delivery suite of Northwick Park Hospital during 1977 in what was originally seen as a pilot study. They were stored flat in polythene bags at −20°C and measured after thawing when time permitted. No attempt was made at either a total collection or a truly random sample; nor was there any particular selection or exclusion of cases. Of 513 placentae collected, 84 were eventually excluded because of gaps in the clinical data, leaving 429 which form the basis of this report.

The measurements were simple and attempted to quantify little more than the qualitative assessment found on the case records.

Weight. This was measured after trimming off the membranes to the placental edge, cutting the cord level with the placental surface, and removing blood clot from the maternal surface.

Diameter. The minimum and maximum diameters were estimated by tape measure.

Cord insertion. The distance between the point of cord insertion and the nearest placental edge was recorded.

Cord length and weight. These were measured, but excluded that length of cord which remained on the baby.

Those measurements allowed a number of derived values to be calculated:

'Area', the geometric mean of the placental diameters was taken as an index of cross-sectional area.

'Shape' or the degree of departure from the circular was estimated by the ratio of maximum to minimum diameters.

'Cord eccentricity' was estimated as the distance of cord insertion divided by mean placental diameter.

'Placental thickness', was crudely assessed as placental weight/'area'^{3}. This estimate was based on the observed relation between weight and 'area' which was linear in the logarithms of these variables.

'Cord thickness' was estimated as cord weight/cord length$^{4/3}$, based on a similar method to that used in estimating placental thickness.

These variables were compared with two simple aspects of fetal growth and well-being: the Apgar score at five minutes after delivery; and standardized birth weight, that is the birth weight corrected for gestational age, sex and maternal parity, height and body weight, on the basis of the standards of Thomson *et al* (1968).

Relations were tested statistically by Kendall's tau correlation coefficient (Kendall, 1970) to avoid making the assumption that variables were normally distributed.

RESULTS

There was no relation between any of the placental variables and the Apgar score of the infant, but 399 (93 per cent) of the infants had an Apgar score of 9 or 10 so that a useful comparison was scarcely possible.

TABLE I

The relation of placental and cord measurements to the standardized birth weight of the infant ($N = 429$)

Variable	Kendall's tau correlation coefficient	Equivalent normal deviate	P
Placental trimmed weight	0·401	12·37	0·001
'Area'	0·324	10·00	0·001
'Thickness'	−0·057	−1·75	N.S.
'Shape'	−0·023	−0·69	N.S.
Cord 'eccentricity'	0·024	0·73	N.S.
Cord insertion	0·107	3·29	0·001
Cord 'thickness'	0·102	3·15	0·002

N.S. = Not significant.

The relations between these variables and the standardized birth weight are given in Table I. Placental weight, and its closely associated 'area' showed an expected measure of correlation, but there is no support for the belief that 'thickness' is related to fetal weight.

There was slight evidence of a weak positive correlation between standardized birth weight and both 'cord insertion' and 'cord thickness' both of which were themselves weakly correlated with placental 'area'. When the effect of placental 'area' was allowed for, the correlation with cord insertion disappeared and that with cord thickness was reduced to a level of significance of only five per cent.

Thirty-nine (9·1 per cent) of the placentae were circumvallate, and the presence of this condition was not related to the two measures of fetal health and growth examined.

DISCUSSION

This preliminary study, designed to see which variables of gross placental morphology deserved closer observation, was deliberately a low cost exercise and therefore relatively crude.

Many technical criticisms of the study could be made: for example, that the Apgar score is a notably insensitive measure of the fetal well-being at birth; and that the birth weight standards of Thomson *et al* (1968) which were based on a homogeneous Scottish population were not necessarily appropriate for the heterogeneous population of Harrow. Those criticisms are fair, but it is excessively unlikely that such a resoundingly negative result would be much changed by sophisticating the description of the infant. Investigations over many years have linked cord eccentricity with a variety of objective complications from abortion (Hathout, 1964) to premature delivery (Brody and Frenkel, 1953) and fetal growth retardation (Busch, 1972). Most recently, Uyanwah-Akpom and Fox (1977) examined 1000 placentae and could find only a minor association between cord eccentricity and low birth weight which was not statistically significant; we support that conclusion.

After a discussion of placental weight, Fox (1978) concluded that there was "no reason why the practice of routinely weighing the placenta should be continued". We would go further: the hurried recording by busy midwives of information on placental weight and size, umbilical cord length and on the general appearance of the placenta is of no value and could be stopped without loss either to the understanding of the individual case or to future research. Moreover it might lead to the better recording of more important observations such as retroplacental clot and abnormal cord vasculature.

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INDUCTION OF LABOUR—A SIX YEAR REVIEW

BY

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Summary

A retrospective survey of surgical induction of labour has shown that induced patients had a lower perinatal mortality rate and a lower Caesarean section rate than non-induced women.

THE past three years have seen a reaction in the obstetrical world against surgical induction of labour (Leading Article, 1974; O'Herlihy and McDonald, 1975; Bonnar, 1976; Chalmers *et al*, 1976; Leeson and Smith, 1977). The reputed dangers are the delivery of an immature baby who fails to survive the neonatal period and the possibility of an increased incidence of Caesarean section (Liston and Campbell, 1974; Blacow *et al*, 1975). Many doctors, including some who previously advocated a high induction rate, appear to have been converted to interfering with nature as little as possible (Baird, 1976). Before this pendulum swings too far it would be well to remember the many advantages induction has brought to the practice of obstetrics. This paper reports the results of surgical induction of labour at the Coombe Hospital in the years 1972 to 1977 with especial reference to perinatal mortality and Caesarean section rates.

PATIENTS

In the six years 1972 to 1977, 44 143 patients over 28 weeks gestation were delivered in the hospital. Labour was surgically induced in 15 496 (35 per cent). The indications for the procedure were postmaturity (47 per cent), pre-eclampsia or hypertension (20 per cent), abnormal weight gain (16 per cent), diabetes (2·6 per cent), antepartum haemorrhage (2·0 per cent), poor obstetric history (1·5 per cent) and miscellaneous (10 per cent) including fetal growth retardation, rhesus isoimmunization, a history of threatened abortion and twins. Social reasons accounted for less than 2·5 per cent of the indications for induction. Postmaturity was defined as a pregnancy that had gone beyond 280 days of amenorrhoea.

METHODS

After the patient had an enema, the fore waters were ruptured and an oxytocin infusion started. We put into 1 litre of 5 per cent dextrose 10 units of oxytocin for primigravidae and 2·5 units for multiparae. The infusion was invariably started at a rate of 20 drops/minute and was then increased hourly by 10 drops/minute until uterine contractions were felt every three minutes, or until an infusion rate of 60 drops/minute had been reached. For primigravidae the infusion was then left at this rate, but in multiparae the infusion strength was, if necessary, increased to 10 units of oxytocin/l of 5 per cent dextrose and the infusion was again run at 20 drops/minute increasing by 10 drops hourly to 60 drops/minute as a maximum. All the induced and non-induced patients were treated in the same delivery suite. Fewer than 5 per cent of patients in either group were monitored continuously by cardiotocography.

RESULTS

Table I (the figures refer to the number of babies) shows that the induced patients had a higher percentage of normal vaginal delivery, a lower incidence of Caesarean section and a much lower perinatal death rate than the non-induced population. Because the non-induced patients might be expected to give birth to most of the babies who died of prematurity or congenital abnormality, it is not reasonable to accept a direct comparison between the two groups. To make the two groups more comparable, I have excluded from the perinatal mortality calculations those babies delivered before 36 weeks, those perinatal deaths due to gross fetal abnormality and amongst the induced patients those babies who died before induction. For the purpose of comparing the incidences of Caesarean section, I have removed those patients delivered before 36 weeks, those who had Caesarean sections for breech presentation or placenta praevia, and those who had elective Caesarean sections. With these corrections there were 38 perinatal deaths amongst the induced patients giving a perinatal mortality rate of 2·45/1000 as against a rate of 7·32/1000 for the non-induced patients. The incidence of Caesarean section was also lower in those patients who had been induced. There were nine babies who died of respiratory distress syndrome. Their details are given in Table II.

TABLE I

Mode of delivery and perinatal deaths (figures refer to number of babies)

	Induced		Non-induced	
	No.	per cent	No.	per cent
Spontaneous	12 578	80·5	22 083	76·0
Forceps/Vacuum	2400	15·4	3519	12·1
Caesarean sections	490	3·1	2504	8·6
'Corrected' Caesarean sections	481	3·1	1214	4·5
Breech	162	1·0	983	3·4
Perinatal deaths	118 (7·6/1000)		765 (26·2/1000)	
'Corrected' perinatal deaths	38 (2·45/1000)		203 (7·32/1000)	

DISCUSSION

Our results show that surgical induction of labour was associated with a lower perinatal mortality rate and a lower Caesarean section rate than was the spontaneous onset of labour. There were two points in our obstetric practice which may have accounted for this. The first was our attitude to fetal maturity and the second our technique for using oxytocin.

We regarded term as the onset at 40 weeks not the onset of 38 weeks of amenorrhoea. The two-week interval between 38 and 40 weeks makes little practical difference to an infant but if the mother is a month out in her dates the two-week interval between 34 and 36 weeks may be crucial. If one considers the nine babies who died of respiratory distress syndrome (Table II), four mothers did not have strong indications for induction and in the first of the two patients listed as being induced for weight loss the mother, who was only seen for the first time at 36 weeks, gave us the wrong information. However, except for the last patient in Table II,

TABLE II

Gestational age, indication for induction, weight, autopsy findings and Farr score of nine babies dying with respiratory dysfunction

Gestational age (weeks)	Indication for induction	Weight (g)	Autopsy result	Gestational age according to Farr* (weeks)
38	Antepartum haemorrhage	2920	Atelectasis	—
38	Fetal growth retardation	2041	HMD	—
38	Pre-eclampsia	3232	„	—
37	Nephritis	2693	„	—
40	Latent diabetes	2693	„	37
38	Discomfort	2750	„	—
40	Maternal weight loss	2268	„	34
40	Maternal weight loss	3090	„	—
39	Long journey to hospital	2500	„	39

HMD = hyaline membrane disease.

* Farr *et al* (1966).

all were delivered before July 1974. Before 1972 the incidence of surgical induction in our hospital had never exceeded 16 per cent. This figure doubled in 1972 and there may have been a tendency to induce patients slightly earlier than hitherto. In July 1974 we became more aware of the risk of unsuspected prematurity and the need for a strong indication for induction before 40 weeks. This knowledge and measurements of the lecithin-sphingomyelin ratio in amniotic fluid may explain why no baby has died from respiratory distress after the induction of labour since 1974.

We used oxytocin more cautiously than others (Turnbull and Anderson, 1968), four hours elapsing before the dosage achieved reached three times the starting dose. The low incidence of section and fetal distress amongst our induced patients seemed to justify our policy.

Finally, an induced labour means a fully supervised labour. This may confer considerable advantage on a community where patients like to stay at home in early labour and tend to come to hospital only when far advanced in the first stage of labour, a time when unsuspected fetal growth retardation may present as fetal distress (Pearson, 1975).

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PLASMA HCG LEVELS IN PATIENTS WITH BLEEDING IN THE FIRST AND SECOND TRIMESTERS OF PREGNANCY

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Summary

The prognostic value of maternal plasma human chorionic gonadotrophin (hCG) determinations, measured by a specific β-subunit radioimmunoassay, was studied in 188 patients with bleeding between 6 and 20 weeks gestation. The patients were arranged into different subgroups according to ultrasound findings as well as clinical and histopathological evidence. In threatened abortion with successful outcome (50 per cent of all patients studied), the weekly mean hCG values were normal or even slightly elevated. In patients with a blighted ovum, the first hCG level measured was in the normal range in 34 per cent of the patients. In patients who aborted embryo with former life signs, hCG values were generally normal before the abortion. The hCG levels were usually low in patients with incomplete abortion and ectopic pregnancy. An initially subnormal level of hCG was associated with a poor outcome of pregnancy in 92 per cent of patients. If the first hCG level was within the normal range the outcome of pregnancy was favourable in 79 per cent of patients.

THREATENED abortion occurs in 16 to 21 per cent of all pregnancies (South and Naldrett, 1973). The frequency of spontaneous abortions has been traditionally estimated to be between 10 and 15 per cent. Modern specific and sensitive methods have made it possible to measure trophoplastic hormones in very early pregnancy; by using the specific β-subunit radioimmunoassay of serum human chorionic gonadotrophin (hCG), the frequency of miscarriages in early pregnancy has been observed to be as high as 25 to 30 per cent (Braunstein *et al*, 1977). Clinical examination alone often fails to predict the outcome of pregnancy and complementary information is needed. During the last few years, the radioimmunoassays of serum or plasma oestradiol, progesterone, human placental lactogen (hPL), hCG and α-fetoprotein (AFP) have largely replaced urinary hormone measurements. Ultrasound examinations have also proved reliable and informative in early pregnancy complications (Jouppila and Piiroinen, 1975; Robinson, 1975).

Earlier methods of hCG measurements did not discriminate between LH and hCG, but the new radioimmunoassay methods are based on recognition of the β-subunit of hCG and measure only hCG and not LH. HCG may be detected in maternal serum as early as 7 to 10 days after ovulation (Kosasa *et al*, 1973). Braunstein *et al* (1978) reported hCG levels in early pregnancy failures, but no evaluation of hCG determinations in different types of early pathological pregnancies has been reported. We now do so with a prospective study of hCG determinations in patients with bleeding at 6 to 20 weeks of pregnancy.

PATIENTS AND METHODS

The study groups comprised 188 patients admitted to hospital with uterine bleeding between 6 and 20 weeks gestation. The control group consisted of 118 women admitted between 6 and 20 weeks gestation for legal termination. After routine clinical examination an ultrasound scan was performed on all patients, using a Kretz Combison apparatus equipped with grey scale and supplemented by Doppler, A-mode and Time motion techniques. The main purpose of the scan was to identify the group of patients with threatened abortion and positive evidence of fetal life and to distinguish them from patients with a blighted ovum, an incomplete abortion, and ectopic or molar pregnancies using the criteria of Donald *et al* (1972) and Robinson (1975). Estimates of gestational age were based on menstrual history or evidence obtained at ultrasound scans. A total of 148 patients had one scan, 28 patients had two and 12 patients had three scans before a definite diagnosis was made. The pregnancies were followed until delivery or abortion; careful clinical and histopathological examinations were performed to confirm the diagnoses made by ultrasound.

Blood samples were taken into heparinized tubes at each ultrasound examination. The plasma was separated immediately and stored at −20°C until analysed.

Plasma hCG was measured using a kit for the plasma hCG β-subunit supplied by NIAMDD, NIH, Bethesda (Vaitukaitis *et al*, 1972).

The samples were diluted 1:50 with phosphate buffered saline (pH 7·3) containing 1 per cent bovine serum albumin. Fifty μl volumes of the diluted plasma were taken in duplicate for assay. Standards (2nd International Standard, WHO) were prepared in male serum diluted as above. The sensitivity of the assay with the above sample dilution was about 0·4 IU/ml. Above this concentration no cross-reactivity with physiological levels of LH (in male, female midcycle and female postmenopausal plasma) was observed. The samples yielding zero-values were reassayed without sample dilution. In this case, the sensitivity of the assay was about 20 mIU/ml. The intra-assay variation of the hCG determinations was below ± 5 per cent and the interassay variation was ± 13 per cent.

HCG values showed a normal Gaussian distribution when the data were expressed logarithmically. Statistical calculations were performed after transforming the data in this way. Statistical comparisons were made using Student's t-tests.

RESULTS

There were 188 patients in this study and the outcome of pregnancy was unsuccessful in 95 (50 per cent). There were 103 patients in whom fetal life was detected by ultrasound at the time of threatened abortion; 93 had a delivery at term and 10 aborted a fresh or live fetus. There were eight tubal and two twin pregnancies.

The normal range of hCG values constructed from weekly estimates, is given in Figure 1.

Threatened abortion with successful outcome (93 patients)

The weekly mean levels of hCG were in most cases slightly above the mean control values (Fig. 2), especially between 8 and 11 weeks gestation. The differences were significant ($p < 0·01$) at 9 and 10 weeks gestation and almost significant ($p < 0·05$) at 8 and 11 weeks gestation. HCG values below normal levels were observed in six patients (7 per cent). A rising trend in hCG levels was observed in most of the 26 patients on whom consecutive determinations were made (Fig. 3), all values being within or slightly above the normal range. The hCG values measured in both twin pregnancies were inside the normal range at 8, 10 and 14 weeks gestation.

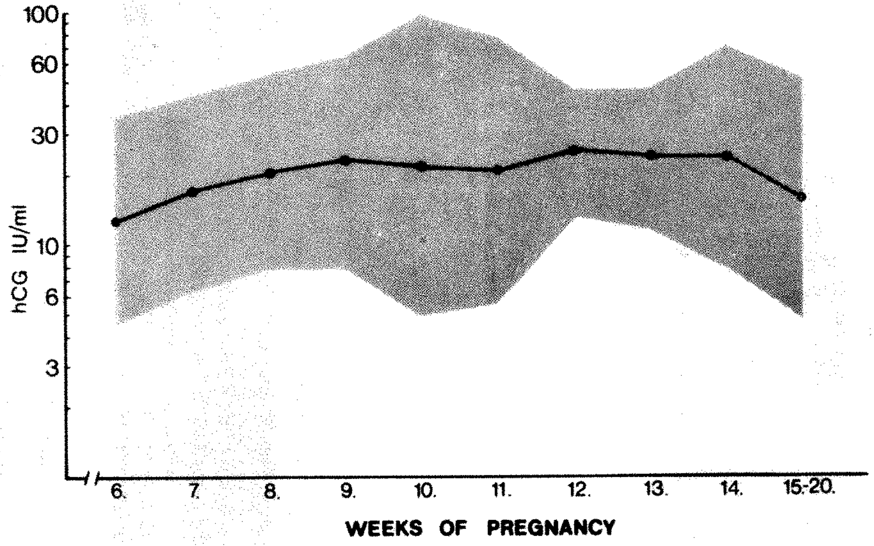

FIG. 1

Plasma hCG levels in the control patients between 6 and 20 weeks gestation. The solid line indicates the mean hCG level and the shaded area the 95 per cent confidence limits. The calculations were performed after logarithmic transformation of the data. The number of individual determinations is 6 to 10 on weeks 6 and 11 to 20, and 16 to 24 on weeks 7 to 10.

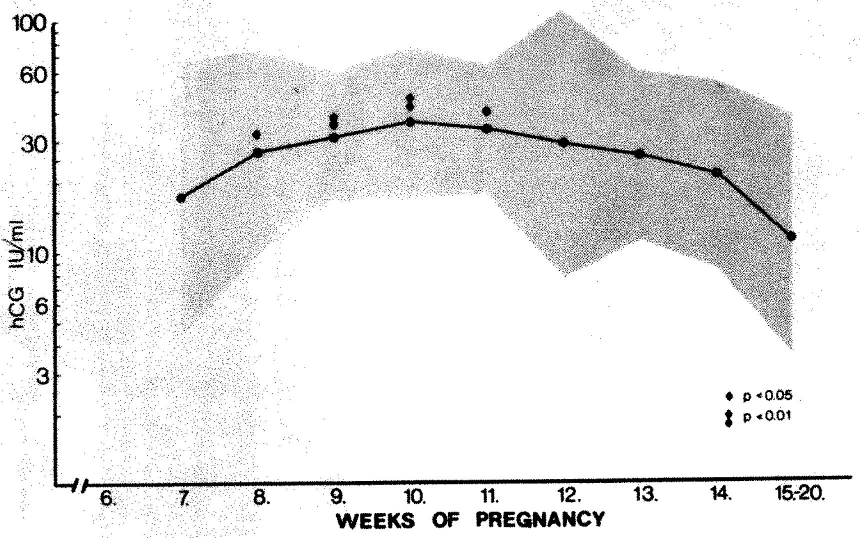

FIG. 2

Plasma hCG levels between 7 and 20 weeks gestation in patients with threatened abortion and a progressed pregnancy of favourable outcome. The solid line indicates the mean hCG level and the shaded area the 95 per cent confidence limits. The calculations were made as in Figure 1. The number of individual determinations is 6 to 11 on weeks 12 to 20, and 13 to 30 on weeks 7 to 10. The diamonds indicate levels significantly higher than in controls (see Fig. 1).

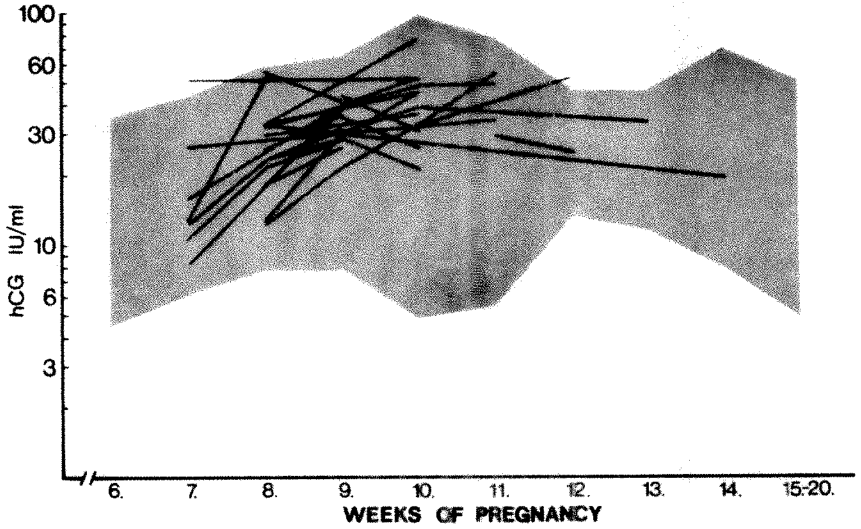

FIG. 3

Consecutive determination of hCG levels in 26 patients with threatened abortion and a pregnancy of successful outcome. The shaded area represents the 95 per cent confidence limits of the hCG distribution of controls (see Fig. 1).

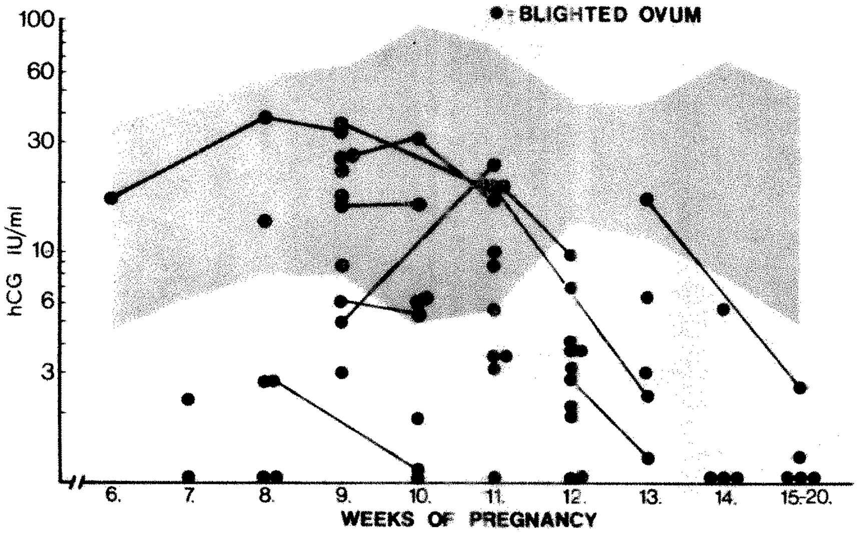

FIG. 4

Individual hCG values in the group of patients with a blighted ovum. The shaded area represents the 95 per cent confidence limits of the hCG distribution of the controls (see Fig. 1).

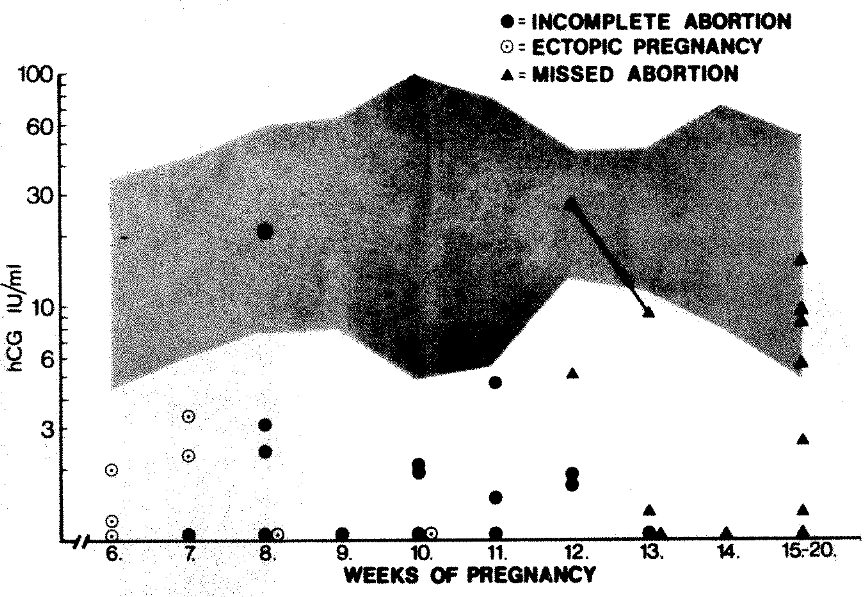

FIG. 5

Individual hCG values in the groups of patients with incomplete abortion, missed abortion and ectopic pregnancy. The shaded area represents the 95 per cent confidence limits of hCG levels for the controls (see Fig. 1).

Spontaneous abortion of a live or fresh fetus (10 patients)

The first hCG level measured at the time of threatened abortion was inside the normal range in eight patients. Four patients aborted at or before 12 weeks and six aborted after 12 weeks. In eight patients, an hCG determination was obtained 1 to 14 days before miscarriage, and it was within the normal range in six of these.

Blighted ovum (50 patients)

The hCG values in 19 patients of this group (38 per cent) were inside the normal range on at least one occasion (Fig. 4). The first value observed was inside the normal range in 17 patients (34 per cent). Most of the hCG levels decreased in consecutive determinations.

Incomplete abortion (14 patients), missed abortion (12 patients), ectopic pregnancy (8 patients) and molar pregnancy (1 patient)

In all the patients with an incomplete abortion the hCG levels were very low and below the normal range (Fig. 5).

In the missed abortion group there were five patients with the first hCG value inside the normal range (42 per cent) despite the absence of signs of fetal life at 12 and 17 weeks gestation (Fig. 5).

In ectopic pregnancies the levels were between 0·5 to 4·0 IU/ml and well below the normal range (Fig. 5) except for one patient who had a normal hCG level (21·1 IU/ml) at 8 weeks. At laparotomy a tubal pregnancy with an 8 cm fetus was found.

In the one patient with a molar pregnancy, the level of hCG was very high, 129 IU/ml at 14 weeks gestation.

During the whole observation period, the weekly mean hCG levels in patients whose pregnancy had an unfavourable outcome were lower ($p < 0{\cdot}001$) than in patients whose pregnancy ended in a successful delivery.

Of the 78 patients with an hCG levels below the normal range at first examination, the outcome was unsuccessful in 72 (92 per cent). If, on the other hand, the first value was inside the normal range (110 patients) the pregnancies ended in term delivery in 79 per cent (87 patients).

DISCUSSION

The practically equal distribution of pregnancies of favourable and unfavourable outcomes agrees with earlier reports (Johansen, 1970). We also confirmed that abortion occurs in only 10 per cent of patients showing ultrasound signs of fetal life at the time of first bleeding (Jouppila, 1977).

HCG values which were below the normal range at the onset of bleeding predicted very well (92 per cent reliability) an unsuccessful outcome of pregnancy. This reliability is higher than that observed previously by Kunz and Keller (1976) (75 per cent) and Jovanovic *et al* (1978) (79 per cent). On the other hand Nygren *et al* (1973) found an hCG level below 10 IU/ml to signify later abortion in 97 per cent of patients. Crosignani *et al* (1974) found that assay of both hCG and hPL in patients with threatened abortion were reliable indicators in 94 per cent.

If the first hCG measurement was within the normal range, we found the outcome of pregnancy to be a term delivery in only 79 per cent of patients. This relatively poor predictive value is in agreement with the 71 per cent obtained by Kunz and Keller (1976) and highlights the common occurrence (38 per cent) of normal hCG levels in cases of blighted ovum. These anembryonic pregnancies have a tendency to continue trophoblastic hormone production for a relatively long time. In addition, in patients who aborted a live fetus, the first hCG measurement was within the normal range, and in the majority the secretion of hCG continued to be normal until the abortion occurred. Thus the miscarriage of a living fetus does not seem to be due to an abnormality of trophoblastic tissue and its hormone production. The fact that the abortions in this group mostly occurred after 12 weeks gestation may signify cervical incompetence or faulty placental attachment as the reason for the miscarriage.

Our finding of relatively high hCG values in threatened abortions with successful outcome was surprising and has not been previously observed. The differences between these and the corresponding control values were significant between 8 and 11 weeks gestation. The high levels can possibly be explained by increased leakage of trophoblastic hormones to the maternal circulation at the time of the bleeding episode and uterine contractions. A first hCG value below the normal range was observed in only 7 per cent of this group.

The most uniform and reliable information from the hCG levels was obtained in patients with an incomplete abortion. In ectopic pregnancies, our low hCG values are in agreement with several observations (Braunstein *et al*, 1978; Saxena and Landesman, 1978) but one ectopic pregnancy with a normally developed fetus at 8 weeks had a normal level of hCG.

Between 6 and 9 weeks gestation, signs of fetal life cannot always be detected by ultrasound. If this failure to detect fetal life is associated with hCG levels below the normal range an unsuccessful outcome of pregnancy is likely. But the prognostic value of normal hCG levels is reduced by the fact that hCG levels were often within the normal range in the cases of live abortion and blighted ovum.

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CONCENTRATIONS OF THE PROSTACYCLIN METABOLITE, 6-KETO-PROSTAGLANDIN $F_{1\alpha}$, IN AMNIOTIC FLUID DURING LATE PREGNANCY AND LABOUR

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Summary

Concentrations of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin, PGI_2) have been measured in amniotic fluid obtained during late pregnancy and labour. Samples taken at amniotomy during spontaneous labour contained a significantly greater concentration of 6-keto-$PGF_{1\alpha}$ than samples taken at amniotomy before the onset of labour ($p < 0{\cdot}01$). There was no correlation between the level of 6-keto-$PGF_{1\alpha}$ in amniotic fluid before labour and gestational age ($p > 0{\cdot}1$). It is suggested that prostacyclin may have a functional role in the mechanism of parturition in man.

IT is now well accepted that prostaglandins play an important part in the progression, and perhaps the onset, of labour in man (Liggins *et al*, 1977; Turnbull *et al*, 1977). Reliable measurements of prostaglandin concentrations in blood are extremely difficult because of the low endogenous levels (Béguin *et al*, 1972; Dray *et al*, 1975; Mitchell *et al*, 1978*c*) and production by platelets (Smith and Willis, 1970). Hence many investigators have been attracted by the lack of significant prostaglandin synthesizing or metabolizing activity in amniotic fluid (Keirse, 1975; Keirse and Turnbull, 1975) and concentrations of E and F prostaglandins in amniotic fluid during late pregnancy and labour have now been established (Karim and Devlin, 1967; Keirse and Turnbull, 1973; Hillier *et al*, 1974; Keirse *et al*, 1974; Keirse *et al*, 1977*a*). Recently, it has been shown that the prostaglandin endoperoxides, the immediate precursors of these prostaglandins, can also be converted into at least two other very active compounds namely thromboxane A_2 (Hamberg *et al*, 1975) and prostaglandin I_2 also known as prostacyclin (Moncada *et al*, 1976). Both compounds have short half lives and are rapidly converted to the more stable metabolites respectively, thromboxane B$_2$ (TXB$_2$) and 6-keto-prostaglandin F$_{1\alpha}$ (6-keto-PGF$_{1\alpha}$). Measurements of the concentrations of these compounds in biological fluids and tissues during parturition could contribute greatly to an understanding of the possible roles of their unstable precursors in this process. In the rat, 6-keto-PGF$_{1\alpha}$ has already been shown to be a major product formed by uterine homogenates (Fenwick *et al*, 1977).

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The development of a radioimmunoassay for TXB$_2$ has already allowed us to determine changes in its concentration in amniotic fluid with labour (Mitchell *et al*, 1978*d*). More recently the development of a radioimmunoassay for 6-keto-PGF$_{1\alpha}$ (Mitchell, 1978) has allowed us to measure its levels in amniotic fluid during late pregnancy and parturition in sheep (Mitchell *et al*, 1978*b*) and now in man with the results presented here.

PATIENTS AND METHODS

Amniotic fluid was obtained from 73 women who were subsequently delivered of normal healthy infants. Full and informed consent to participation in the study was obtained from all women, although the amniotic fluid samples were obtained in the course of routine clinical care and not for the purpose of research. All samples were free from contamination with blood or meconium. Samples were taken (a) by abdominal amniocentesis to assess fetal pulmonary maturation by measurement of amniotic fluid phospholipid concentrations before induction of labour between weeks 35 to 39 of pregnancy (n = 9); (b) at amniotomy for induction of labour between weeks 37 to 43 of pregnancy (n = 33) and (c) at amniotomy during spontaneous labour between 37 and 43 weeks (n = 31). Amniotic fluid was stored at −20°C until assay.

The 6-keto-PGF$_{1\alpha}$ levels were measured by a specific radioimmunoassay that has been fully described and evaluated elsewhere (Mitchell, 1978). The assay includes both extraction and chromatographic procedures before the final radioimmunoassay which uses an iodinated histamine-conjugated tracer for 6-keto-PGF$_{1\alpha}$. The lower limit of sensitivity of the assay was 1·5 pg/tube and the intra-assay coefficient of variation was 8 per cent.

Statistical differences were calculated using the Mann Whitney Rank Sum Test.

RESULTS

Amniotic fluid taken by amniotomy during spontaneous labour contained significantly greater concentrations of 6-keto-PGF$_{1\alpha}$ (1230 ± 191 pg/ml; mean ± SEM) than fluid obtained by amniotomy before the onset of labour (444 ± 92 pg/ml) ($p < 0{\cdot}01$). The method of collection of amniotic fluid before labour did not influence measured levels ($p > 0{\cdot}1$; Fig. 1) although the number of samples obtained by amniocentesis was small. This latter conclusion held even if the two highest levels in the amniotomy group were excluded and also when samples collected only between 37 to 39 weeks were considered, which reduced bias caused by differences in gestational ranges. Before labour there was no correlation ($p > 0{\cdot}1$) between gestational age and levels of 6-keto-PGF$_{1\alpha}$ in amniotic fluid collected by amniocentesis or by amniotomy.

During labour there was no evidence of a correlation between cervical dilatation and amniotic fluid 6-keto-PGF$_{1\alpha}$ concentrations (Fig. 2). Statistical analysis using, for example, the Spearman Rank correlation is not valid because of so many 'tied' observations (11 ties at 4 cm dilatation). There was no significant difference (Mann Whitney Rank Sum Test)

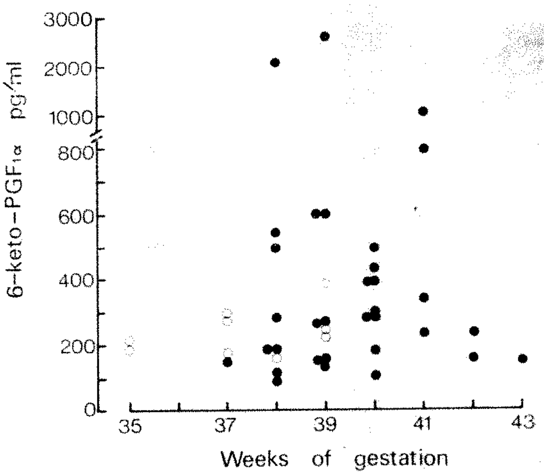

FIG. 1

Concentrations of 6-keto-prostaglandin F$_{1\alpha}$ in amniotic fluid obtained by amniocentesis (○) and by amniotomy (●).

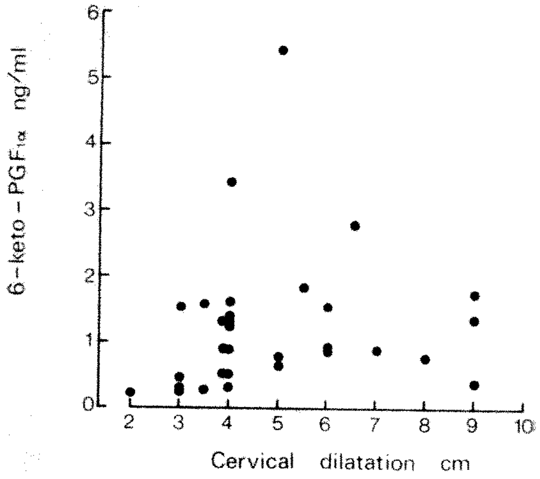

FIG. 2

Relationship between the concentration of 6-keto-prostaglandin $F_{1\alpha}$ in amniotic fluid and the degree of cervical dilatation during spontaneous labour.

between levels in samples collected between 2 and 4 cm dilatation and those between 5 to 9 cm ($p > 0{\cdot}1$) or between 2 to 5 cm and 6 to 9 cm ($p > 0{\cdot}1$) suggesting no trend in concentrations as the cervix increasingly dilates.

DISCUSSION

These results have demonstrated the existence of immunoreactive 6-keto-PGF$_{1\alpha}$ in amniotic fluid during late human pregnancy and parturition. Furthermore, it has been shown that levels are elevated in spontaneous labour compared with levels in late pregnancy before the onset of labour, in keeping with our previous findings for prostaglandins (PG) E and F, 13,14-dihydro-15-keto-PGF (PGFM) and arachidonic acid (Keirse and Turnbull, 1973; Keirse *et al*, 1974; Keirse *et al*, 1977*a*; Keirse *et al*, 1977*b*). Although only few samples obtained by amniocentesis were studied, it appears that 6-keto-PGF$_{1\alpha}$ is not subject to rapid release by amniotomy as demonstrated for PGF, PGFM and TXB$_2$ (Mitchell *et al*, 1976; Mitchell *et al*, 1978*d*). This difference in a possible mechanism for the control of production would be consistent with our previous suggestion that the production of prostanoid compounds may be controlled differentially by the activities of the enzymes responsible for their conversion from prostaglandin endoperoxides (Mitchell *et al*, 1978*d*).

The major source of prostaglandins in amniotic fluid remains controversial with the decidua (Karim and Devlin, 1967; Gustavii, 1977) and fetal membranes (MacDonald *et al*, 1974; Keirse and Turnbull, 1976; Mitchell *et al*, 1978*a*) both receiving considerable attention. It has been suggested (Willman and Collins, 1978) that umbilical arteries produce considerable quantities of 6-keto-PGF$_{1\alpha}$. Our own results (Mitchell *et al*, in preparation) strongly favour the amnion as a major site for its production. Since fetal membranes are also a major source of PGE, this would argue in favour of a central role for this structure in the mechanism of human parturition.

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SERUM FOLLICLE STIMULATING HORMONE LEVELS IN HUMAN PREGNANCY

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Summary

We describe a specific radioimmunoassay for follicle stimulating hormone (FSH) and the finding of immunoreactive FSH in human pregnancy sera. A total of 394 serum samples was taken from 204 normal pregnant women at different stages of gestation. The FSH levels remained constant throughout pregnancy and no rise was associated with the late first trimester elevation of human chorionic gonadotrophin (hCG).

THE human placenta is the site of production of large quantities of sex steroids, particularly the oestrogens. In the presence of high levels of circulating sex steroids, one would expect, by means of the negative feedback, marked suppression of secretion of the anterior pituitary gonadotrophins. However, due to problems of cross reactivity both in bioassay and radioimmunoassay systems, little is known concerning pituitary gonadotrophin secretion in human pregnancy. Although the cross reactivity of anti-sera with both luteinizing hormone (LH) and human chorionic gonadotrophin (hCG) remains a problem and still obviates the measurement of LH in pregnancy, specific antisera have now been raised against follicle stimulating hormone (FSH) which do not significantly cross react with hCG. As a result, FSH can now be measured in the presence of the large amounts of hCG found in human pregnancy sera. Thus pituitary activity, with regard to FSH secretion, can now be studied in the human subject during the gestation period.

We describe the finding of immunoreactive FSH in human pregnancy sera and report the levels of FSH detected during the course of normal pregnancy. A comparison is made with the levels of FSH in serum in non-pregnant women.

METHODS

Assay of FSH and LH

The FSH was assayed using a double antibody radioimmunoassay technique.

The diluent used was a 0·01 M phosphate buffer containing 0·9 per cent sodium chloride and 0·5 per cent bovine serum albumin (Armour Pharmaceutical Co. Ltd.) at pH 7·2. The incubation volume was 1 ml per tube.

The tracer was prepared from the Medical Research Council preparation 71/333 and was iodinated using carrier-free Iodine ^{125}I (Radiochemical Centre, Amersham, England). Iodination was performed using the chloramine T method (Greenwood *et al*, 1963). Specific activity ranging from 200 to 300 μCi/μg was obtained over a series of 10 iodinations.

The specific hCG-adsorbed anti-FSH serum used in the assay is denoted M93 and was a generous gift from Dr W. R. Butt. The antiserum had been prepared in New Zealand White rabbits by an immunization technique described by Lynch and Shirley (1975). Scatchard analysis showed that this antiserum had a binding constant of $1 \cdot 0 \times 10^{11}$ l/mol. In the assay it was used at a final dilution of 1 in 4 million.

The reference standard antigen used in the assay was the Medical Research Council preparation denoted FSH 68/39 and the results are expressed as ng/ml of serum in terms of the reference preparation used.

The incubation time for the assay was five days. All samples were assayed in duplicate.

The second antibody consisted of a solution of donkey antirabbit serum (Wellcome Laboratories Ltd.) diluted 1 in 10 in 0·01 M phosphate buffer also containing 0·07 M E.D.T.A. and 2·5 per cent normal rabbit serum. To each assay tube was added 100 μl of the second antibody solution and after mixing of the contents of each tube, the assay was incubated for a further 24 hours.

The assay tubes were then centrifuged for one hour at 1500 *g* and the supernatant separated from the precipitate. All tubes were then counted in a Hewlett Packard gamma counter. The count from the precipitate was then expressed as a percentage of the total count per assay tube.

The hCG levels were assayed in a similar

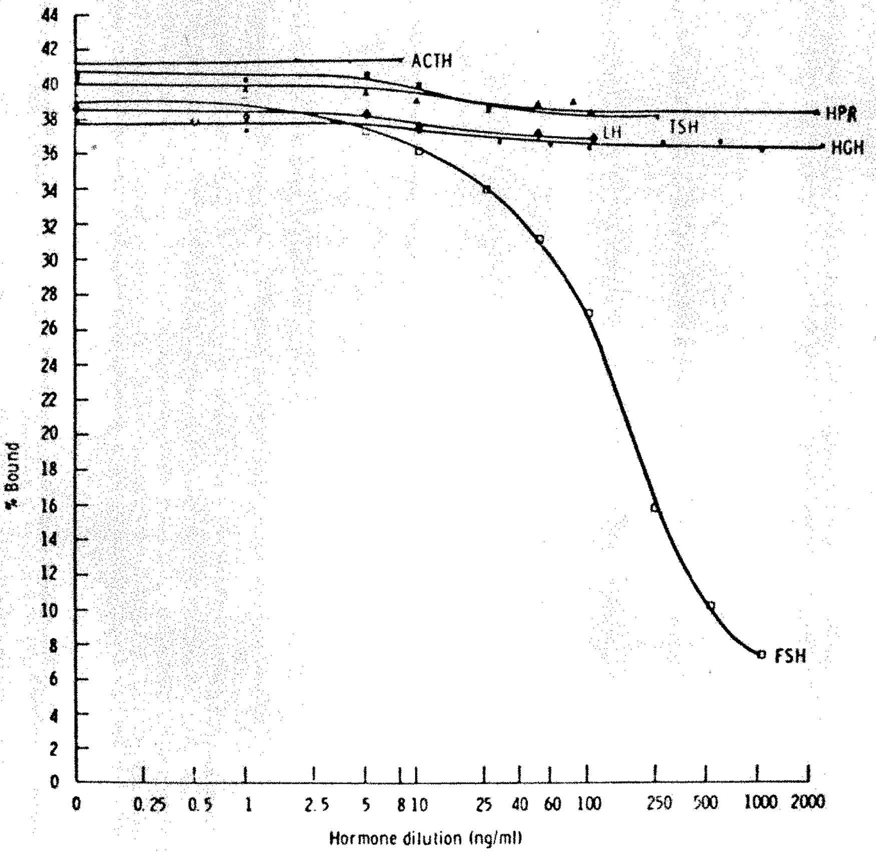

FIG. 1

Cross reaction study showing no significant cross-reaction between luteinizing hormone (LH), thyroid stimulating hormone (TSH), human growth hormone (HGH), adrenocorticotrophic hormone (ACTH) and human prolactin (HPR) with M93 anti-FSH serum. Inhibition of binding by the FSH standard 60/40 is also shown.

manner. The tracer was obtained already iodinated as hCG-^{125}I from Eurotope Services Ltd. The standard antigen used in the assay was obtained from the Medical Research Council and denoted 2nd International Reference Preparation. The assay was also incubated for five days and precipitation of the bound fraction was achieved in the same manner as in the FSH assay.

Characteristics of the FSH assay

The double antibody radioimmunoassay for FSH in pregnancy sera would appear to be specific. The standard curve measured between 10 and 500 ng/ml in terms of the 68/39 standard used. Service volumes of 100 μl were used in the assay.

Cross reaction studies using other pituitary hormones were performed. No significant cross reaction was seen using 250 ng of LH (MRC 68/40), 250 ng of thyroid stimulating hormone (MRC 1st International Reference Preparation), 10 ng of adrenocorticotrophic hormone (NIH ≠ 8B), 200 ng of human growth hormone (WHO 1st International Reference Preparation and 2000 ng of human prolactin (NIH — VLS2) (Fig. 1).

The non-specific binding, namely binding in the absence of unlabelled FSH and antibody, was unaltered by the addition of either 100 μl of pregnant sera or 10 000 μg of the 2nd International Reference Preparation of hCG to each tube. However, a cross reaction between the hCG and the anti-FSH serum was important to exclude. Addition of doses of hCG to a maximum of 11 000 ng per tube showed a short biphasic inhibition of 11·6 per cent from the initial binding with a maximum inhibition of

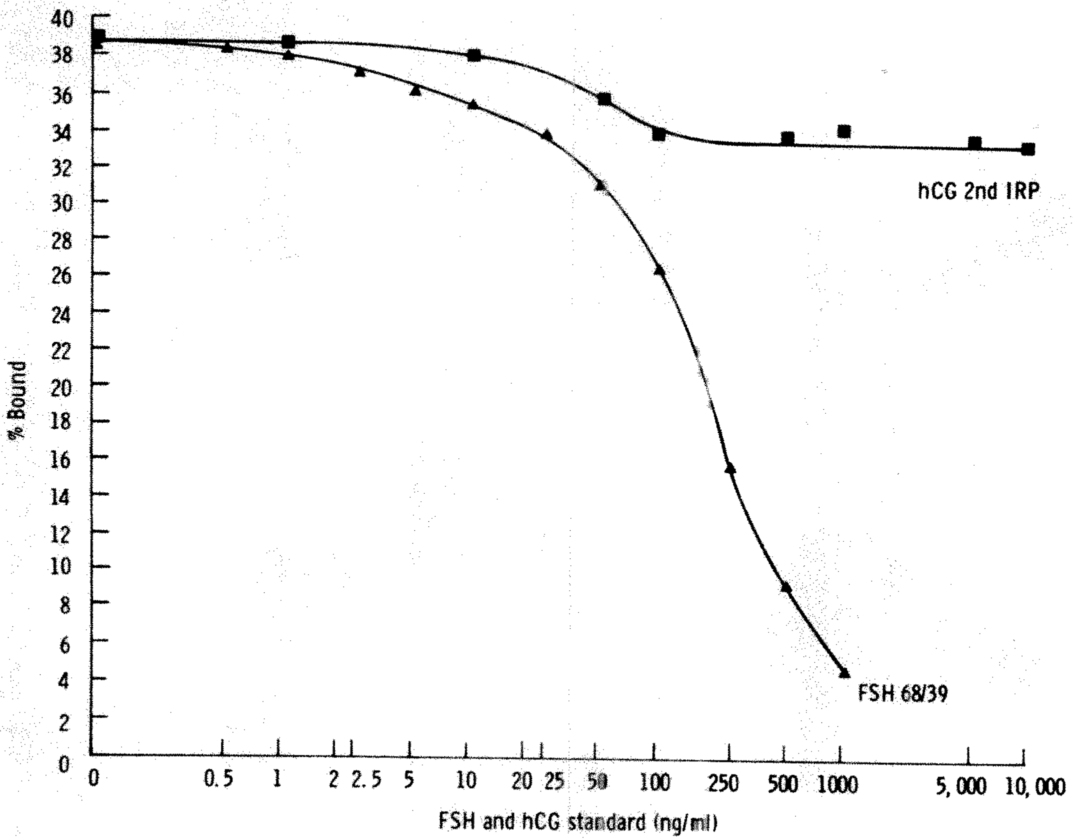

FIG. 2

Cross reaction study between hCG and FSH using anti-FSH serum (M93).

binding when 100 ng/ml of hCG was added (Fig. 2). Increasing amounts of hCG produced no further inhibition. This suggests that there still exists in the antiserum a population of antibodies which do cross react with hCG and which are adsorbed out by the addition of 100 ng of hCG to each tube. Greater quantities of hCG (up to 10 000 ng/ml) did not cause further inhibition of binding. This could be explained by the presence of two populations of antibodies, one which reacted with the antigen components common to hCG and FSH and which can be displaced by hCG and the other specific for FSH. As the inhibition of binding produced by 100 ng of hCG did not match the inhibition of binding produced either by FSH itself or by the pregnancy sera, one can conclude that the difference represents true inhibition of binding by FSH contained in the pregnancy sera. Sera from a pregnant woman, a premenopausal woman, and a postmenopausal woman were serially diluted and the assay results with the standard curve, were subjected to logit-log transformation. Parallelism was demonstrated between the standard curve and all three serum samples (Fig. 3).

The inter-assay variation obtained by using 10 serum pools in 10 assays produced a coefficient of variation of 10·4 per cent.

Patients

There were 204 healthy women with normal

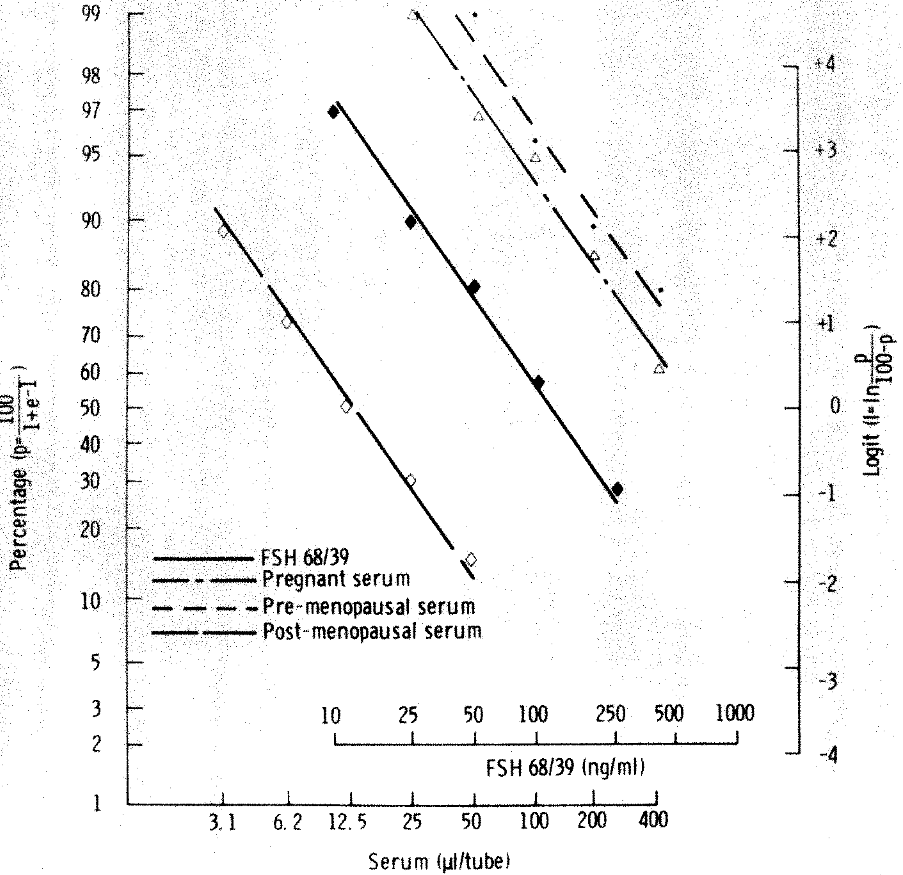

FIG. 3

The parallel inhibition of binding of iodinated FSH to FSH antiserum (M93) by FSH standard as well as by serial dilutions of postmenopausal serum, premenopausal serum and the serum of a pregnant woman.

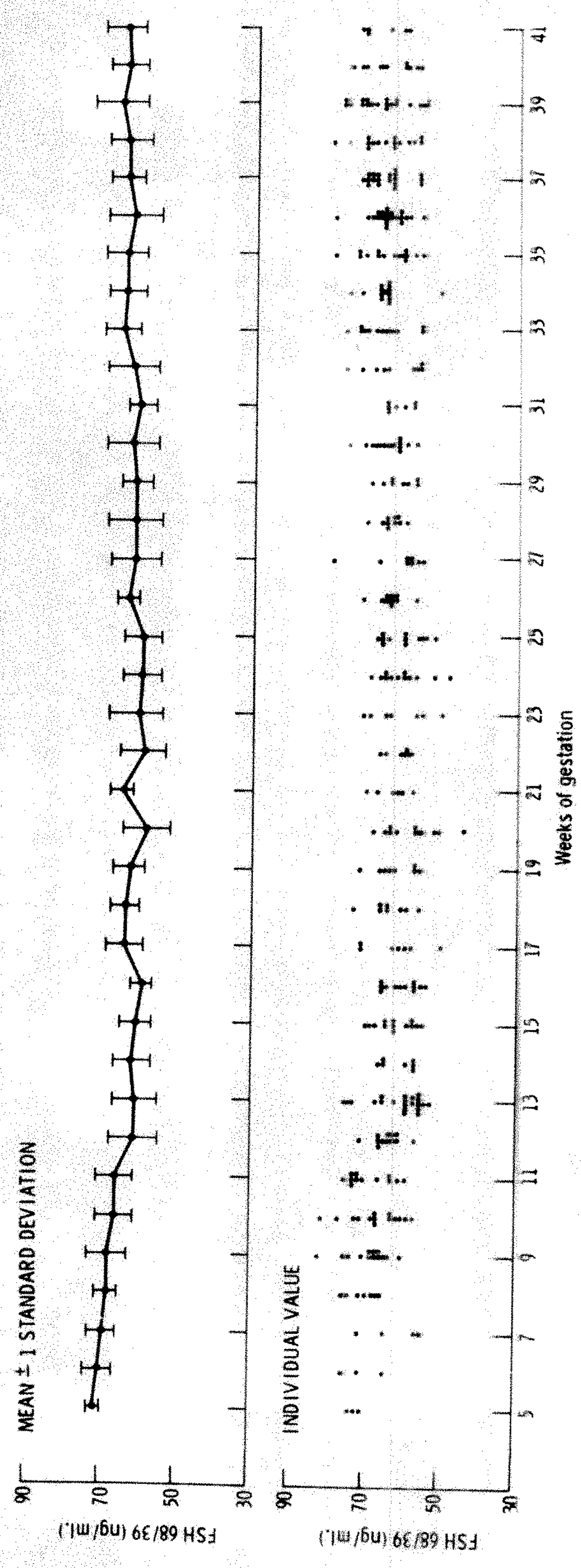

FIG. 4

Levels of FSH in serum during normal pregnancy. Above, the solid line represents the mean values at different weeks of gestation ± 1SD. Below, the dots represent individual values.

pregnancies of which 72 were primiparae and 132 were multiparae and whose ages ranged from 16 to 40 years. The routine physical examination and laboratory tests done in pregnancy gave normal results. The menstrual cycle prior to conception was reported as normal and all the women were sure about the date of their last menstrual period. Peripheral venous blood was obtained at random gestational ages from the 5th to the 41st week. The number of samples obtained from each subject varied from 1 to 8 at different stages of gestation. A total of 394 samples was assayed for FSH; 72 of these samples, from 15 subjects, were also assayed for hCG.

For comparison with the levels of FSH found in peripheral venous blood in the non-pregnant state, single samples were obtained one day pre-operatively from 54 normal women who were to undergo sterilization operations for social reasons. Thirty of these samples were from women in the follicular phase of a normal 26 to 30 day menstrual cycle. All these women were menstruating normally and ovulation was

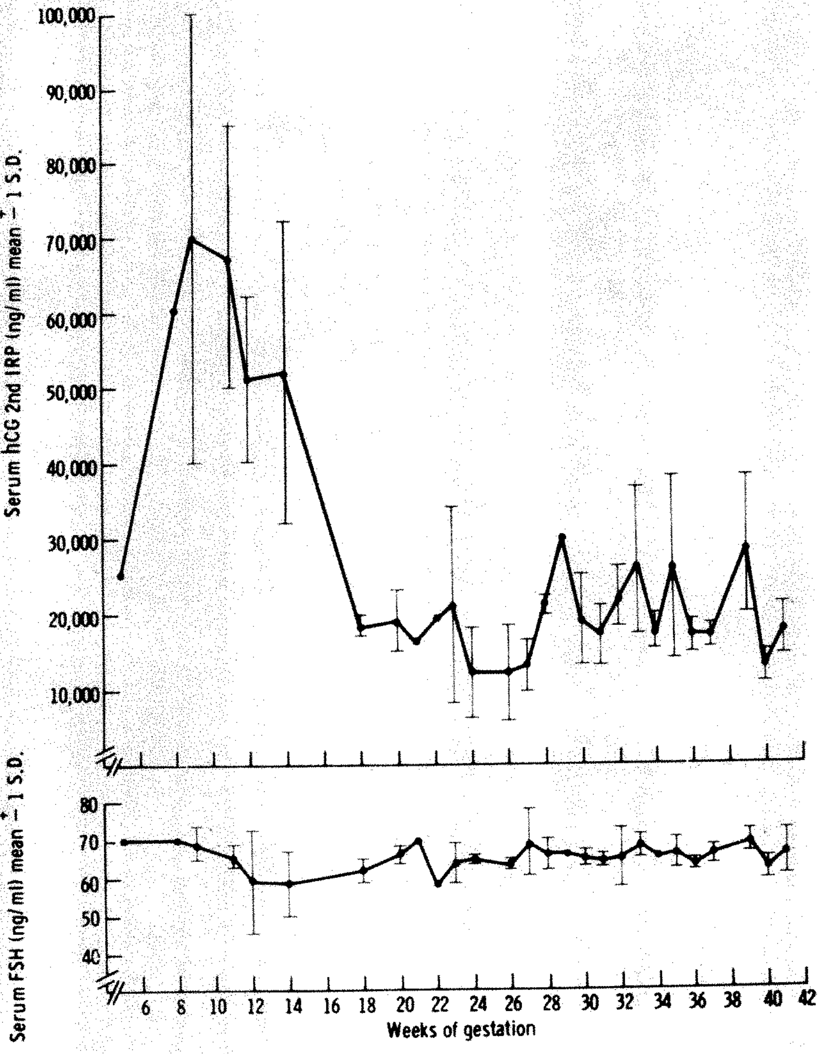

FIG. 5

Serum hCG and FSH levels in 15 normal women during pregnancy. The mean ± ISD of the hCG levels are depicted above and those of FSH below.

proved in the luteal phase subjects by endometrial biopsy which was done routinely at the time of operation. A further 19 normal women taking a combined oral contraceptive were sampled once during the third week of the first month of therapy.

At each sampling, 5 ml of venous blood was taken from an antecubital fossa vein. The blood was allowed to clot at 4°C, the serum was separated by centrifugation and stored at −20°C until assayed. All patients were attending the City Hospital in Nottingham.

RESULTS

FSH levels in pregnant and non-pregnant women

The levels of FSH in serum in a total of 394 samples from 204 women during the course of a normal pregnancy are shown in Figure 4. FSH was measurable in all of them and remained very constant throughout gestation. The FSH and hCG levels (mean±ISD) in 15 of the pregnant women are shown in Figure 5. The levels of FSH in pregnancy remain constant and unlike the levels of hCG show no significant change in early pregnancy.

The levels of FSH (mean±ISD) measured in 100 women in the first trimester of pregnancy produced a mean level of 64±6 ng/ml. In the 30 women in the follicular phase and in the 24 women in the luteal phase, the levels of FSH were 125±64 ng/ml and 60±69 ng/ml respectively, while in the 19 women during the third week of combined oral contraceptive therapy, the mean FSH level in serum was 19·9±21 ng/ml. These are shown in Figure 6. Thus the levels of FSH in pregnancy most closely resemble the levels seen in the luteal phase of a normal ovulatory cycle. However, the range of values seen in the non-pregnant subject was much wider than that observed in pregnancy, although some of the higher values in both the non-pregnant groups may represent a midcycle FSH peak.

COMMENT

The presence of FSH in the urine of pregnant women was first described by Lyons *et al* (1953) and Vignes *et al* (1954 and 1955). Both groups of workers used an alcohol extraction and a bioassay method utilizing rat follicular maturation. Butt *et al* (1957) suggested that the urine of pregnant women contained a substance with the same biological and chemical properties as that extracted from the urine of postmenopausal women. Crooke and Butt (1959), using a rat ovarian augmentation bioassay, studied for the first time the pattern of FSH excretion throughout normal pregnancy. However, Albert and Derner (1960) argued strongly that the bioassay changes said to be due to FSH in extracted human pregnancy urine were due to an intrinsic FSH-like activity of hCG itself and they did not believe in the presence of a pituitary gonadotrophic hormone in pregnant urine.

With the recent development of highly specific radioimmunoassay methods for pituitary and placental gonadotrophins, the question of the pituitary gonadotrophin activity during pregnancy again came under investigation with the publication of several different studies. Midgley (1967) reported the absence of measurable quantities of serum FSH in pregnancy, while Faiman *et al* (1968) and Hanson *et al* (1970) demonstrated the presence of relatively high concentrations of FSH in pregnancy sera. Jaffe *et al* (1969) were the first to describe FSH during a normal cycle and resultant pregnancy in one subject. Parlow *et al* (1970) studied another patient during an ovulatory cycle and resultant pregnancy, observing a depression of serum FSH levels to below the limits of detection. Czygan *et al* (1972) found that FSH values were as high as in the follicular and luteal phase during the early few weeks of the pregnancy following therapeutic induction of ovulation. However, a review of the studies published to date shows that those workers who have found FSH in measurable amounts in pregnancy sera (Faiman *et al*, 1968; Zarate *et al*, 1973; Ylikorkala *et al*, 1976) agree that the levels of FSH remain very constant throughout pregnancy.

The most striking feature of our study is the constancy of the levels of FSH measured in serum during the course of normal pregnancy in a large number of women. The constancy was apparently already achieved at five weeks after the last menstrual period. However, this constancy has been shown to occur even sooner after conception by Jaffe *et al* (1969), Parlow *et al* (1970) and Czygan *et al* (1972). The constancy compares strikingly with the much wider range

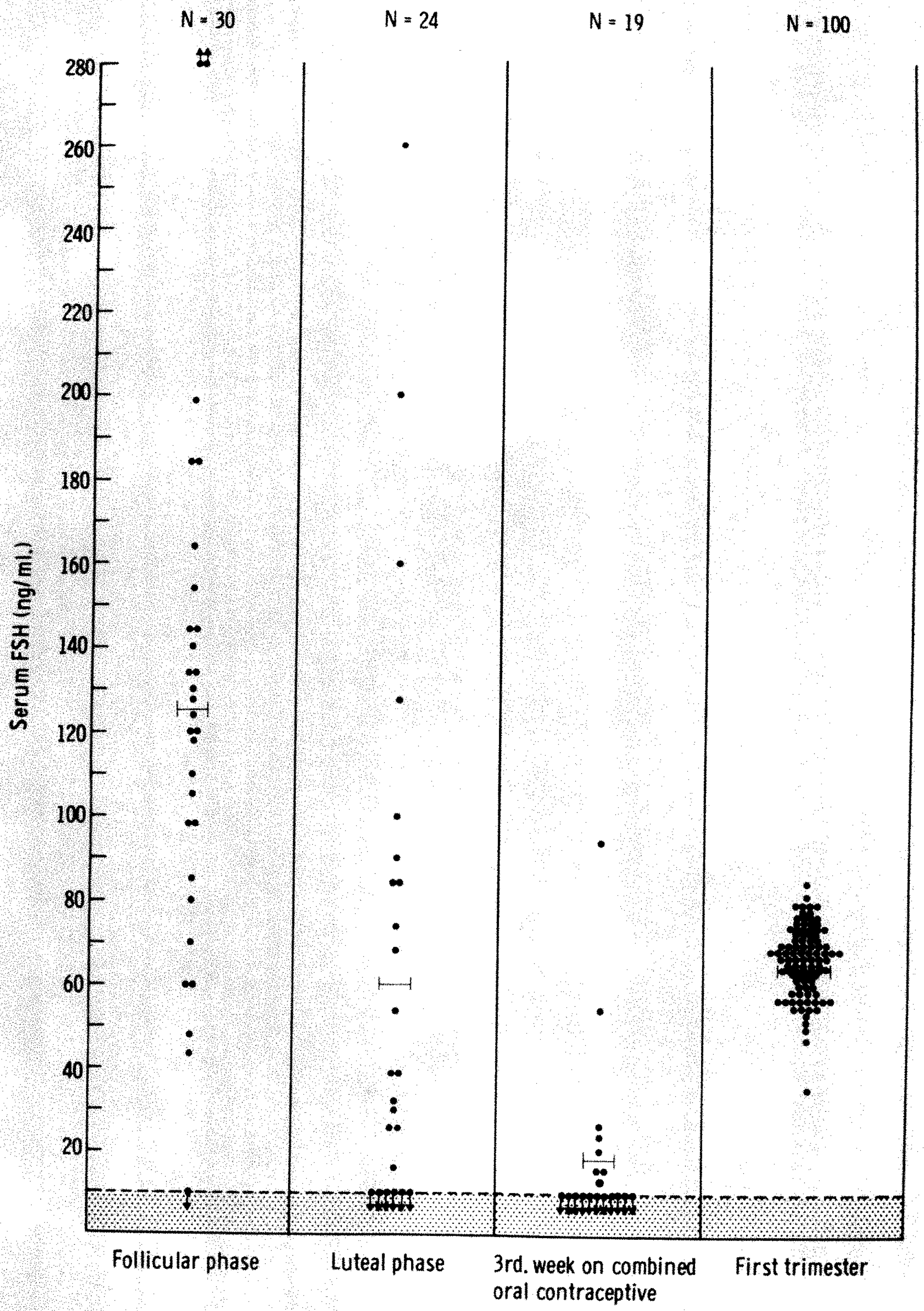

FIG. 6

A comparison between FSH levels in serum in different phases of the normal menstrual cycle, during oral contraceptive therapy and in normal first trimester pregnancy. The mean levels in each group are signified by the horizontal bars.

of FSH values seen during the non-pregnant follicular and luteal phases of the menstrual cycle.

It may be difficult to compare our absolute values of serum FSH in pregnancy with the values found by other investigators due to differences in the reference standard and antiserum used in the assay system. However, most of the available data on FSH levels in pregnancy were shown to be in the range of that seen in the luteal (Mishell *et al*, 1974) or the follicular phase of the menstrual cycle (Czygan *et al*, 1972).

Unchanging levels of a hormone in any assay system always suggest the possibility of some non-specific interference with the assay itself. The characteristic first trimester peak of hCG in serum (Mishell *et al*, 1963) was not accompanied by any changes in levels of FSH. Secondly, the inhibition of binding by the pregnancy sera was greater than that seen when very large amounts of hCG standard were added to the system. Other hCG preparations have been shown to contain FSH-like activity (Saxena and Rathman, 1971) which may support the suggestion that the minor degree of inhibition of binding seen is due to antigenic contamination. If this is the case, more inhibition should occur when the amount of hCG standard is increased but this was not so. The finding of parallelism between serial dilutions of post and premenopausal sera when compared to pregnancy sera also suggests that the assay system is not detecting hCG.

Further support for the concept of the presence of FSH in pregnant sera, is the evidence of continuing follicular maturation in the ovary during gestation (Nelson and Greene, 1958). Why ovulation does not occur in pregnancy is uncertain but it may be that the unchanging levels of FSH unlike those seen in the normal menstrual cycle, together with the absence of a 'mid cycle' peak may not allow full follicular maturation and follicular rupture to occur. There may also be profound alteration in target organ receptor concentration and affinity in pregnancy. The antagonistic effect on the ovary of the elevated levels of prolactin in pregnancy (McNatty *et al*, 1974) may also interfere with ovarian response to the gonadotrophins.

From the present data we believe that FSH is present in small but measurable amounts in human pregnancy sera.

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RED CELL MASS DURING AND AFTER NORMAL PREGNANCY

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Summary

Red cell mass, derived from plasma volume and haematocrit, was calculated serially in 45 healthy women during pregnancy and 4 to 6 months after delivery. Compared to the non-pregnant, postpartum value there was a reduction of approximately 100 ml at 12 weeks gestation. An increase in red cell mass occurred thereafter: at 36 weeks gestation values 180 ml greater than the non-pregnant were found. Iron and folic acid supplements almost doubled this increment to 349 ml of red cells.

ALTHOUGH there is debate about the magnitude of the change, there is general agreement that red cell mass (RCM) is increased during the second and third trimesters of pregnancy (reviews by Hytten and Leitch, 1971; Lange and Dynesius, 1973; Walters and Lim, 1975). In contrast, data concerning first trimester changes in RCM are conflicting: Caton *et al* (1951) and Berlin *et al* (1953) reported reductions of 300 ml and 200 ml respectively during the first trimester of pregnancy, Paintin (1962) and Blekta *et al* (1970) described an increase of approximately 100 ml and Lund and Donovan (1967) showed no change in RCM when corrected for body weight. Of these studies, three were based on cross-sectional data (Berlin *et al*, 1953; Lund and Donovan, 1967; Blekta *et al*, 1970) and one had first trimester data on only seven patients (Caton *et al*, 1951); the data in the serial study by Paintin (1962) of 20 primigravidae suggests that a number of these women were anaemic at the time the non-pregnant RCM was determined, namely 7 to 8 weeks after delivery (Table I).

The purpose of this study was to measure RCM during pregnancy in 45 women and to compare these values with their RCM determined 4 to 6 months post partum, when any pregnancy effect could reasonably be expected to have ended. The effect of haematinic administration on RCM during pregnancy, not hitherto described, was also studied.

SUBJECTS AND METHODS

Subjects

Forty-eight healthy pregnant women with no adverse medical or obstetric history were recruited from the antenatal clinic before the 12th week of gestation. After the initial investigations at 12 weeks gestation, the women were randomly allocated to either a therapy or non-therapy group; those to receive therapy were given a preparation containing ferrous sulphate, 325 mg, and folic acid, 350 ng, to be taken daily from the 12th week until delivery. The non-therapy group received no haematinic therapy during pregnancy. Three women

TABLE I

Summary of the literature on red cell mass in the first trimester of pregnancy

Author	Type of study	Technique used	Source of non-pregnant data	Non-pregnant red cell mass (ml)	First trimester red cell mass (ml)
Caton *et al* (1951)	Serial	^{55}Fe	30 days post partum	1579 (n=7)	1257 (n=7)
Berlin *et al* (1953)	Cross-sectional	^{32}P	Non-pregnant women	1562 (n=16)	1388 (n=15)
Paintin (1962)	Serial	Evans Blue/Hct.	7 to 8 weeks post partum	1292 (n=18)	1393 (n=20)
Lund & Donovan (1967)	Cross-sectional	?Evans Blue ?^{51}Cr	Non-pregnant women	26·9 ml/kg (n=85)	26–27 ml/kg (n=18)
Blekta *et al* (1970)	Cross-sectional	^{51}Cr	Non-pregnant women	1433 (n=22)	1525 (n=33)

TABLE II

Means and standard deviations of the characteristics of the patients in the study

	Non-therapy group	Therapy group
Number	24	21
Age (years) Mean±SD	27·1±3·4	26·4±3·2
Parity*	14:0°; 8:1°; 2:2°	6:0°; 13:1°; 2:2°
Maternal height (cm) Mean±SD	163·5±4·9	162·6±5·9
Maternal weight (kg) Mean±SD	60·1±8·8	58·0±3·8
Infant weight (g) Mean±SD	3502±232	3470±365
Infant sex	13 Male: 11 Female	12 Male: 9 Female
Infant gestation (days) Mean±SD	282·3±6·1	284·1±6·4

* 0° nulliparous 1° primiparous 2° secundiparous.

from the therapy group were delivered before 37 weeks and were therefore excluded from the study. The essential features of the remaining 45 women and the outcome of their pregnancies are summarized in Table II.

Methods

Haematological indices and plasma volume, were studied in each patient at 12 weeks and 36 weeks gestation and finally 4 to 6 months after delivery (PP2). Haematological indices only were measured at 6 to 8 weeks after delivery (PP1).

The blood samples were always taken between 0900 and 1030 hours; after the women had rested for 15 to 30 minutes in a comfortable sitting position the blood was obtained from an antecubital vein without the use of a tourniquet. All had fasted since the previous evening on each occasion.

Haematological indices were determined on a Coulter counter S calibrated daily using "4C" (Coulter Electronics Ltd.).

Plasma volume was measured using a weighed amount of Evans Blue solution as described by Hytten and Paintin (1963). RCM was derived from the plasma volume and Coulter counter haematocrit using a total body/venous haematocrit ratio of 0·88 (Paintin, 1963).

The significance of differences between results was assessed by applying the paired Student "t" test.

RESULTS

Postnatal values

To determine whether data obtained six weeks postpartum were representative of the non-pregnant state, the haematological indices obtained by Coulter counter analysis at 6 to 8 weeks post partum were compared to those measured 4 to 6 months after delivery. A pregnancy effect was still apparent at 6 to 8 weeks in that the average haemoglobin concentration was lower, the red cell count reduced and the haematocrit lower relative to the 4 to 6 month values whether or not the patients received iron and folic acid supplementation; these differences achieved statistical significance (Table III).

In the group of women given iron and folic acid both the mean cell volume and mean cell haemoglobin were significantly higher suggesting that the iron induced macrocytosis (Taylor and Lind, 1976) was not reversed by 6 to 8 weeks after delivery (Table III). For subsequent comparisons, therefore, the data obtained 4 to 6 months after delivery were used as the non-pregnant values.

Pregnancy changes

Plasma volume. The change in this series were comparable to those previously described (Hytten and Paintin, 1963). By 12 weeks gestation plasma volume had risen by approximately 220 ml and by 36 weeks by approximately 1100 ml in both groups (Table IV).

Haematological indices. These are summarized in Table V and confirm those previously described (Taylor and Lind, 1976). The changes in haematocrit are particularly important because of their effect upon calculated red cell mass. By 12 weeks gestation this value had decreased

TABLE III

Comparison of haematological indices at 6 to 8 weeks postpartum with 4 to 6 months postpartum

Investigation	Non-therapy group		*Significance level of differences	Therapy group		*Significance level of differences
	PP1	PP2		PP1	PP2	
White cell count ($\times 10^9$/l) Mean ± SD	7·27 ±2·38	5·64 ±1·01	$p < 0{\cdot}0005$	8·36 ±1·90	6·34 ±1·53	$p < 0{\cdot}0001$
Red blood cell count ($\times 10^{12}$/l) Mean ± SD	4·493 ±0·338	4·688 ±0·309	$p < 0{\cdot}0125$	4·370 ±0·169	4·621 ±0·238	$p < 0{\cdot}0005$
Haemoglobin concentration (g/dl) Mean ± SD	12·69 ±0·92	13·30 ±0·77	$p < 0{\cdot}0005$	13·03 ±0·45	13·42 ±0·66	$p < 0{\cdot}005$
Haematocrit (l/l) Mean ± SD	0·3787 ±0·0289	0·3936 ±0·0233	$p < 0{\cdot}01$	0·3880 ±0·0123	0·3971 ±0·0190	$p < 0{\cdot}0125$
Mean cell volume (fl) Mean ± SD	84·1 ±3·8	83·7 ±3·1	NS	88·4 ±3·3	85·7 ±2·2	$p < 0{\cdot}0005$
Mean cell haemoglobin (pg) Mean ± SD	28·23 ±1·45	28·39 ±1·06	NS	29·86 ±1·20	29·00 ±0·77	$p < 0{\cdot}0005$
Mean cell haemoglobin concentration (g/dl) Mean ± SD	33·47 ±0·93	33·75 ±0·68	NS	33·59 ±0·66	33·63 ±0·69	NS

PP1 6 to 8 weeks post partum.
PP2 4 to 6 months post partum.
* Student t test (paired).

TABLE IV

Plasma volume and red cell mass in the pregnant and non-pregnant state

Investigation	Non-therapy group			Therapy group		
	Non-pregnant	12 weeks	36 weeks	Non-pregnant	12 weeks	36 weeks
Plasma volume (ml) Mean ± SD	2340 ±233	2540 ±298	3478 ±408	2336 ±214	2577 ±246	3384 ±399
Red cell mass (ml) Mean ± SD	1240 ±146	1138 ±136	1420 ±181	1256 ±141	1159 ±136	1605 ±221

TABLE V

Haematological indices. All values are expressed as Means ± SDs

Investigation	Non-therapy group			Therapy group		
	Non-pregnant	12 weeks	36 weeks	Non-pregnant	12 weeks	36 weeks
White cell count ($\times 10^9$/l)	5·64 ±1·01	6·89 ±1·71	10·24 ±3·39	6·34 ±1·53	8·06 ±·171	10·02 ±1·70
Red blood cell count ($\times 10^{12}$/l)	4·688 ±0·309	4·008 ±0·247	3·880 ±0·304	4·621 ±0·238	4·109 ±0·227	4·119 ±0·246
Haemoglobin concentration (g/dl)	13·30 ±0·77	12·03 ±0·70	11·07 ±0·84	13·42 ±0·66	12·06 ±0·57	12·66 ±0·81
Haematocrit (l/l)	0·3936 ±0·0233	0·3515 ±0·0226	0·3311 ±0·0232	0·3971 ±0·0190	0·3539 ±0·020	0·3666 ±0·020
Mean cell volume (fl)	83·7 ±3·1	86·2 ±3·6	85·0 ±5·3	85·7 ±2·2	86·0 ±3·3	88·8 ±2·9
Mean cell haemoglobin (pg)	28·39 ±1·06	30·07 ±1·16	28·65 ±2·00	29·00 ±0·77	29·43 ±1·03	30·76 ±1·24
Mean cell haemoglobin concentration (g/dl)	33·75 ±0·68	34·23 ±1·13	33·46 ±0·82	33·63 ±0·69	34·05 ±1·08	34·50 ±0·82

TABLE VI

Comparison of the calculated effect of the increase in plasma volume on haematocrit with the actual haematocrit recorded at 12 weeks gestation

	Non-therapy		Therapy	
	Non-pregnant	12 weeks gestation	Non-pregnant	12 weeks gestation
Plasma volume (ml) Mean ± SD	2340 ±233	2540 ±298	2336 ±214	2577 ±246
*Calculated haematocrit (l/l) Mean ± SD	—	0·3760 ±0·0409	—	0·3738 ±0·0236
Actual haematocrit (l/l) Mean ± SD	0·3936 ±0·0233	0·3515 ±0·0226	0·3971 ±0·0190	0·3539 ±0·0200

* Each subject's calculated haematocrit =

$$\frac{\left(\frac{\text{NPPV}}{1-\text{Hct}}\right)-\text{NPPV}}{\left[\left(\frac{\text{NPPV}}{1-\text{Hct}}\right)-\text{NPPV}\right]+\text{PPV}}$$

Where

NPPV = non-pregnant plasma volume
PPV = 12 weeks plasma volume
Hct = non-pregnant haematocrit

significantly below the non-pregnant level in both groups (p <0·0005 for both groups). In the non-therapy group there was a further decrease at 36 weeks gestation but this was not the case for those given iron. It could be argued that the reduction in haematocrit at 12 weeks was a 'dilutional' effect from the increased plasma volume alone and unrelated to any change in RCM. However, if the proportional effect of each subject's increase in plasma volume at 12 weeks gestation on their non-pregnant haematocrit is calculated, a mean haematocrit of 0·37 (l/l) is found in both groups, whereas the actual determined value was 0·35 (l/l) (Table VI). There is therefore a genuine reduction in haematocrit by 12 weeks gestation.

RCM. Those patients who were subsequently given iron and folic acid showed an average reduction in red cell mass of 97 ml by the 12th week while the other group showed a fall of 102 ml (Table IV). Both of these reductions are statistically significant (p <0·0005 and p <0·0025 respectively). The difference between the two groups is not statistically significant. This first trimester reduction was greater for primigravidae (Table VII). The effect of giving iron and folic acid was virtually to double the incremental rise in RCM; the average increase at 36 weeks gestation was 349 ml in the therapy group while the non-therapy group had an average increase of 180 ml (Fig. 1).

DISCUSSION

The two previous serial studies of changes in RCM during pregnancy (Caton *et al*, 1951; Paintin, 1962) used 30 day and 7 to 8 week postpartum values respectively as 'non-pregnant' data. Our results suggest that this was probably

TABLE VII

Red cell mass at 12 weeks gestation by parity

Group	Primigravidae (n = 20)		Multiparae (n = 25)	
Gestation	Non-pregnant	12 weeks	Non-pregnant	12 weeks
Red cell mass (ml) Mean ± SD	1262 ±152	1125 ±147	1236 ±116	1166 ±130
* Significance of differences		p <0·0005		p <0·0025

* Student t test (paired).

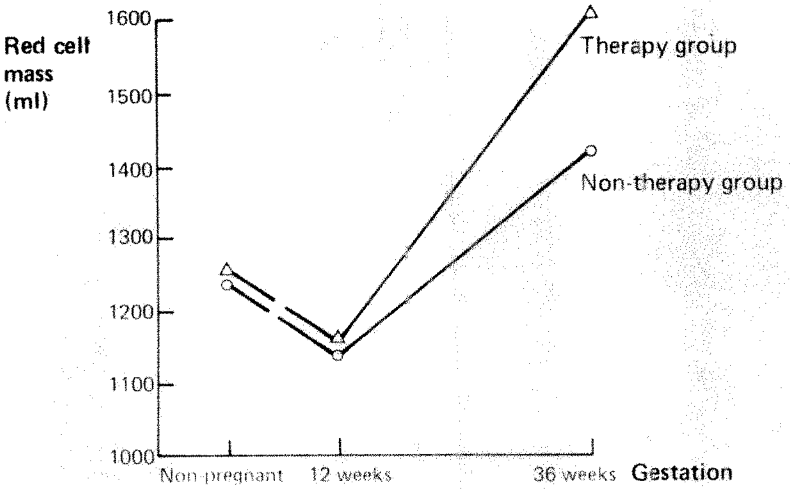

FIG. 1

Red cell mass changes during pregnancy.

too short a period; we have found that pregnancy-induced haematological changes were still present some 6 to 8 weeks post partum relative to the 4 to 6 month data. Using these 4 to 6 months postpartum data there is an actual reduction of about 100 ml of red cells by the end of the first trimester. The increases in RCM thereafter are close to those discussed by Hytten and Leitch (1971). These authors suggested a rise of about 250 ml in the absence of iron supplementation and a rise of about 400 to 450 ml in red cell mass when iron supplementation was taken during pregnancy; we found increases of 180 ml and 349 ml in the non-therapy and therapy groups respectively. The slightly smaller average increases in our study may reflect the greater accuracy of measuring haematocrit with a Coulter counter compared with previous techniques. The latter methods overestimate haematocrit since the centrifuged red cell column always contains between 2 and 6 per cent 'trapped' plasma (England *et al*, 1972). There are other inaccuracies due to the inability to obtain a flat seal at the bottom of the haematocrit tube and the relative difficulty in reading the height of the column of red cells.

The reduction in RCM by 12 weeks gestation could be caused by an increase in destruction of red cells, a decrease in their production or a combination of both processes. There is no evidence that red cell destruction is increased in late pregnancy (Pritchard and Adams, 1960) and we have not been able to find any data concerning red cell survival during early pregnancy.

The control of red cell production is probably multifactorial, but one important influence is the hormone erythropoietin. The production of erythropoietin is dependent on the tissue oxygen level in the kidney which, in turn, is governed by a number of independent factors including atmospheric oxygen tension, pulmonary function, cardiac output, RCM and the oxygen affinity of haemoglobin. During the first trimester of normal pregnancy there are changes in cardiac output and the oxygen affinity in haemoglobin which would tend to influence the tissue oxygen levels in the kidney. Cardiac output increases by an average 1 to 2 litres/minute (Walters *et al*, 1966; Lees *et al*, 1967) while renal blood flow is increased by 25 per cent in early pregnancy (Sims and Krantz, 1958). It has been reported that 2,3-diphosphoglycerate (2,3-DPG) production is increased early in pregnancy (McDonald and McDonald, 1977); an increase in 2,3-DPG causes a shift of the oxygen dissociation curve to the right decreasing maternal haemoglobin affinity and thereby facilitating oxygen transfer to the tissues. In the face of an increased blood flow and increased oxygen supply the kidney would be expected to reduce production of erythropoietin. This sequence of events could explain our finding of a reduction in RCM during the first trimester. The factors which control the early reduction and later increase in RCM during pregnancy require further study. The concurrent changes in the iron transport and iron storage systems are at present under investigation.

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TREATMENT OF PRURITUS VULVAE BY MULTIPLE INTRADERMAL INJECTIONS OF ALCOHOL. A DOUBLE-BLIND STUDY

BY

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Summary

A double blind study is described in which 17 patients with intractable pruritus vulvae were treated by multiple intradermal injections of absolute alcohol. In each patient half the vulva was treated with alcohol whilst the other half was injected with water. Treatment with alcohol was significantly better than treatment with water ($p < 0{\cdot}001$).

THE treatment of intractable pruritus vulvae using multiple intradermal injections of absolute alcohol has been described previously (Woodruff and Thompson, 1972; Ward and Sutherst, 1975). The results were encouraging but neither study was a controlled one. There are many causes of pruritus vulvae, and in any patient the degree of irritation varies considerably from time to time with or without treatment. It was considered appropriate, therefore, to evaluate this treatment further by means of a double blind study using the patient as her own control.

PATIENTS AND METHODS

Seventeen patients were treated. They were aged between 26 and 70 (mean 52 years), and had had pruritus for an average of 10 years. Standard therapeutic measures had failed. Vulvectomy had been performed in four patients without benefit.

Vulval biopsy was performed in all patients before treatment. Histological appearances are shown in Table I. There was a variable degree of hyperkeratinization and chronic inflammation, but in no patient was there any epithelial atypia.

After giving informed consent each patient was randomly allocated to one of two groups. In one group the right side of the vulva was treated with alcohol, and in the other group the left side. In each patient the opposite side was injected with sterile water instead of alcohol.

The method has been described in detail elsewhere (Ward and Sutherst, 1975). Under general anaesthesia the anogenital area was marked out with ink in 1 cm squares. At each line intersection between 0·1 and 0·2 ml of either absolute alcohol or sterile water was injected into the deeper dermis (namely 5 to 6 mm below the skin surface).

The study group to which each patient belonged was known only to the theatre nurse. All the operations were performed by myself, and neither myself nor the patient knew which side of the vulva had been treated with alcohol until follow-up examination two months later.

RESULTS

At follow-up, 14 of the 17 alcohol treated areas of skin were free of pruritus, although in each of these patients the skin of the opposite side of the vulva was still itching. In no patient was the pruritus less in the water injected side than in the alcohol treated side.

In two patients symptoms were relieved on both sides, and in one other the pruritus was unaltered by either type of injection.

TABLE I

Histology of vulval skin in 17 patients with pruritus vulvae

Chronic epithelial dystrophy		11
hypertrophic	(7)	
atrophic	(4)	
Changes consistent with scratching		3
Normal skin		3
Total		17

For the 14 patients where the treatments were distinguishable, the sign test (or the normal approximation to the sign test described by Hill, 1977) gives a (2-sided) p value of $<0 \cdot 001$ (for Ho: treatments are equi-effective).

Thirteen of the 14 patients cured on one side were subsequently readmitted for treatment of the opposite side with intradermal alcohol. Eight of these have remained symptom-free since the second treatment (average 2 years, range 0·5 to 4 years). In three patients the pruritus recurred after several months, but it was less severe than before, and has responded to the occasional application of topical steroids. Two women treated early in the study have needed repeat treatments (one has had three, and one has had two bilateral treatments), but they have remained symptom-free for most of the time in between.

Some degree of postoperative discomfort was experienced by all the women, but was relieved by mild analgesics. Mild cellulitis which resolved spontaneously was observed twice, but on no occasion was there any sloughing or necrosis of the skin.

DISCUSSION

Many therapeutic measures have been described for pruritus vulvae caused by benign lesions of the vulval skin. They include the topical application of steroids, antihistamines, and local anaesthetics. Psychotropic drugs such as the tricyclic antidepressants and tranquillizers have also been used. However, standard methods are not always effective. Recourse may then be taken to surgery, though even vulvectomy does not necessarily cure the condition. Four patients in this series had had vulvectomy performed previously.

It used to be believed that many of the chronic epithelial dystrophies were premalignant conditions (Berkeley and Bonney, 1909). Treatment had to take this as well as the pruritus into account and vulvectomy was commonly performed. It is now realised that the risk of malignant change is low, particularly when there is no atypia of the epithelium (Woodruff, 1976). There is, therefore, less need for ablative surgery.

The results of my study indicate that injections of intradermal alcohol can often cure pruritus vulvae. The injections cause temporary destruction of nerve endings in the skin (Woodruff and Thompson, 1972). Sensation is lost for approximately six weeks and when it returns it does so without pruritus. The vicious cycle in which repeated scratching causes the vulval skin to become progressively more traumatized and irritable is, therefore, broken.

Alcohol is well known for its ability to cause skin necrosis. However, complications such as skin blistering (from injecting too superficially) and focal sloughing (from injecting too deeply) can be avoided by directing careful attention to technique. If care is taken, side effects are minimal, and the patient's time in hospital is short.

This treatment could therefore be considered as an alternative to other surgical procedures when simple remedies have failed.

The single patient in the series who did not improve had an interesting history. She had been deserted by her husband 50 years previously after three days of non-consummated marriage, and had remained virgo intacta. She could not understand why a person so clean living should be so afflicted. Vulval skin had been excised twice and the patient had also had numerous vulval biopsies. She had no relatives and lived alone. One gained the impression that complaining of pruritus was the only way she could attend the out-patient clinic and maintain contact with the outside world.

The two patients whose pruritus disappeared on both sides after unilateral treatment with alcohol might represent two spontaneous remissions as they cannot be readily explained otherwise.

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IN VITRO STUDY OF THE POSSIBLE ROLE OF THE INTRAUTERINE CONTRACEPTIVE DEVICE TAIL IN ASCENDING INFECTION OF THE GENITAL TRACT

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Summary

Potentially pathogenic bacteria colonized the mucus coating the tails of both monofilamentous and multifilamentous intrauterine contraceptive devices (IUCD) in 72 (55 per cent) out of 132 *in vitro* tests. This suggests that the IUCD tail may be responsible for the passage of vaginal bacteria into the uterus.

As early as 1931, the presence of a tail attached to an intrauterine contraceptive device (IUCD) was suggested as a pathway for the entry of organisms from the vagina to the sterile uterine cavity (Gräfenberg, 1931). For this reason Gräfenberg designed the ring without a tail. Nevertheless, when Lippes (1963) reintroduced tailed devices, he found no evidence of significant clinical infection. However, when the problem was studied in a well controlled group of 515 patients, Weström *et al* (1976) showed that users of IUCDs had a three-fold increase in the risk of contracting acute salpingitis when compared to non-users. This was true for all the devices used, namely the Lippes loop, Saf-T-coil and Gravigard. As a result of this finding we are currently undertaking a detailed analysis of the role of the IUCD tail in ascending pelvic infections. In this study we report on a laboratory model designed to establish whether bacteria ascend in the mucus which coats the IUCD tail following insertion of the device.

MATERIALS

Test bacteria

The organisms tested included *Escherichia coli*, *Proteus*, *Staphylococcus aureus*, *Corynebacterium vaginale*, *Streptococcus pyogenes*, group B β-haemolytic streptococci, *Bacteroides fragilis* and *Clostridium perfringens* which are all potential pathogens commonly found in the vagina. *Neisseria gonorrhoeae* was also tested.

Mucus

Cervical mucus was aspirated from the cervical canal by syringe at the mid-cycle when the mucus was most copious and was either used immediately or stored at +4°C until required. All samples were cultured and only those yielding small numbers of commensal bacteria were used. Using the *Micrococcus lysodeikticus* assay for lysozyme (Shugar, 1952) it was found that the activity of this enzyme in the samples did not deteriorate on storing.

Tails

The IUCD tails studied were the multifilamentous tail of the Dalkon shield, test modified monofilamentous tails measuring 0·25, 0·3 and 0·5 mm in diameter and the monofilamentous tail of the Gravigard (Copper 7).

METHOD

The cervical mucus was introduced into new 1 ml tuberculin syringe barrels up to the 0·4 ml mark using a double-ended needle. The measured height from the syringe nozzle to the upper surface of the mucus was comparable to the length of the average cervical canal. Mucus was introduced only up to the 0·1 ml mark in experiments using *N. gonorrhoeae* cultures as the anaerobic conditions within larger volumes interfered with survival of gonococci.

The IUCD tails were introduced into the syringes via the nozzle end in order for them to become coated with mucus. To simulate the length of tail remaining in the vagina, 2 cm of each tail was left protruding from the syringe nozzle. Bacteria were suspended in molten blood agar base No. 2 (Oxoid) containing 5 per cent defibrinated horse blood. Cysteine hydrochloride (0·1 per cent) was added in tests using anaerobes. To confirm the size of the inoculum used, viable counts were made by culturing 10 μl and 100 μl of serial decidilutions of the bacterial suspensions. During development of the method, two or three bacterial species were used in each test. The molten medium was added to glass tubes so that the syringe barrel containing the IUCD tail rested with the nozzle tip breaking the surface of the agar when lowered into the tube. The 2 cm of protruding tail thus became embedded in the agar bacterial suspension (Fig. 1). The models were placed in outer glass tubes containing sterile water which were plugged to maintain humidity and incubated at 37°C for five days. Anaerobes were incubated in sealed jars in an atmosphere of 5 per cent CO_2 in hydrogen; similarly gonococci were incubated in an atmosphere of 5 per cent CO_2 in air.

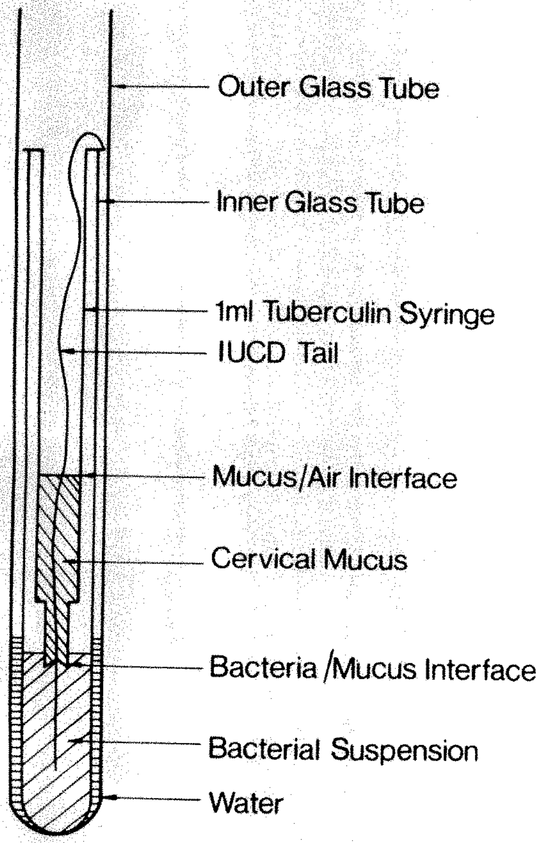

FIG. 1
Experimental model used in our study.

After incubation, the syringe barrel and tail were cut transversely at each 0·1 ml marking on the syringe using a heated scalpel. The pieces of tail were removed and cut into further sections when a combination of bacteria were used. The sections were cultured at 37°C for 48 hours on appropriate media. The presence of bacteria on the sections of tail was recorded in mm from the level of the mucus/air interface.

RESULTS

The experimental model used was designed to determine if bacteria migrate through the layer of mucus coating the outer surface of an IUCD. Two controls were used. First, when the IUCD tail was omitted from the model, bacteria were found not to migrate above the level of the mucus plug in the syringe. In all, 38 negative control tests were made using different combinations of potentially pathogenic bacteria. In the second control system, the mucus plug was omitted so that the tail of the IUCD directly entered the suspension of bacteria in agar. In 22 such tests, bacteria were found not to migrate above the bacterial agar suspension. These

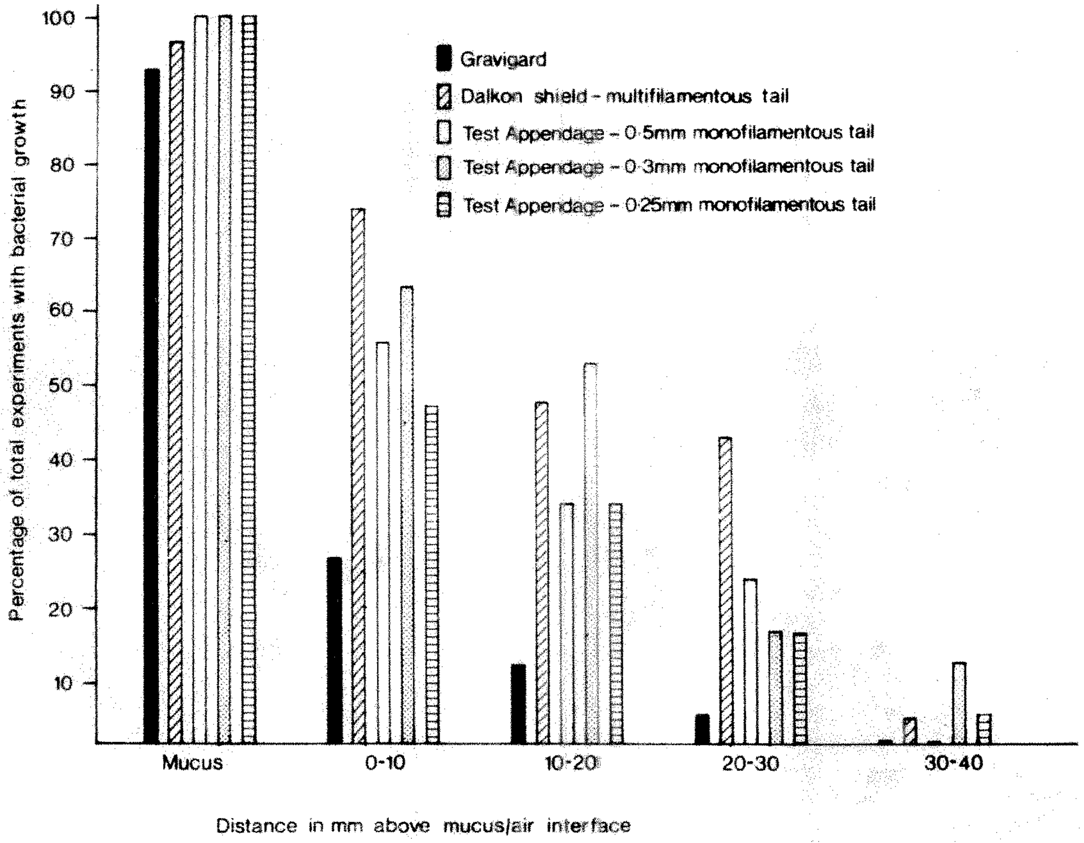

FIG. 2

Bacterial growth in mucus and above mucus/air interface expressed as per cent of total number of experiments per device tail.

controls establish that the recovery of bacteria from the IUCD tail above the mucus/air interface represent the migration of bacteria in the mucus film coating the tail. A total of 135 test models was made. In 132 of these, bacteria were cultured from the cervical mucus whilst in the remaining 3, growth was confined to the length of tail in the agar suspension. In 72 (55 per cent) of the 132 tests which showed bacteria in the cervical mucus, bacteria were cultured from the surface of the tails above the mucus/air interface (Fig. 2). No differences were noted between the multifilamentous and monofilamentous tails but the Gravigard tails yielded significantly fewer cultures from above the mucus/air interface. A probable explanation for this would seem to be that, with the exception of the tails for the Gravigard device, all tails contained a knotted loop at the upper end where they had previously been attached to the IUCD. As the tails were introduced into the syringe via the nozzle, the presence of a knot caused more mucus to be drawn up through the barrel on the tail surface and thus a thicker mucus layer was maintained on the tail surface over the five-day incubation time. Figure 2 shows the bacterial growth which was cultured in the mucus and at lengths of 10 mm intervals above the mucus/air interface. With all types of tails, bacteria were cultured from as high as 20 to 30 mm above the mucus/air interface. At a height of 30 to 40 mm, bacterial growth was recorded on the multifilamentous Dalkon shield, and the 0·3 mm and 0·25 mm monofilamentous nylon tails. No differences were seen between the devices with individual bacterial species (Table I).

TABLE I

Number of tests (maximum of three) showing bacterial growth above the mucus/air interface

	Tail				
			Monofilament nylon		
	Gravigard	Multifilament Dalkon shield	0·5 mm	0·3 mm	0·25 mm
Escherichia coli	2	2	2	3	1
Bacteroides fragilis	0	2	1	2	2
Proteus sp.	1	3	3	3	2
Clostridium perfringens	0	3	3	3	3
Staphylococcus aureus	1	3	2	2	2
Corynebacterium vaginale	0	1	2	1	1
Neisseria gonorrhoeae	0	0	0	0	0
Streptococcus pyogenes	2	3	1	1	1
Group B β-haemolytic streptococci	1	3	1	2	1

DISCUSSION

The normal uterine cavity is sterile although bacteria can be found in the lower half of the cervical canal (Sparks *et al*, 1977*a*). Preliminary studies have shown that the presence of both monofilamentous and multifilamentous tailed IUCDs leads to the presence of small numbers of bacteria in the uterine cavity; however, the cavity remains sterile if the device is without a tail (Sparks *et al*, 1977*b*). The mucus probably plays a role in protecting the uterine cavity from invasion by vaginal bacteria. The cervix contains a local immune system producing secretory IgA which contributes to the normal protective action of the cervical mucus (Rebello *et al*, 1975). That the presence of an IUCD may alter cervical mucus has been shown by an increased sperm penetration of mucus in users of Lippes loop IUCDs (Ansbacher and Campbell, 1971). Although some workers (Barton and Wiesner, 1945; Pommerenke, 1946; Zuckerman *et al*, 1975) reported that cervical mucus inhibited *Staphylococcus aureus*, haemolytic streptococci and *E. coli*, other workers (Rozansky *et al*, 1962) could not demonstrate inhibition. Further, Enhörning *et al* (1970) demonstrated the migration of *Proteus mirabilis* through a column of mid-cycle mucus in 56 per cent of samples tested and the survival of the organisms, but no migration, in a further 30 per cent. This present study confirms that potentially pathogenic bacteria present in the normal vagina will grow in cervical mucus. The introduction of the tails through the mucus in the models results in a covering of mucus adhering to the tail above the mucus/air interface thus mimicking the covering of mucus which occurs on normal insertion of IUCDs. In 72 of the laboratory tests (55 per cent), bacteria were demonstrated growing in this mucus coating. Clearly this represents a potential route along which bacteria might ascend from the vagina to the uterine cavity. In a continuing study of endometrial biopsies obtained at hysterectomy, we found small numbers of bacteria in the uterine cavities in 15 out of 17 patients who had tailed IUCDs whilst 5 patients with IUCDs without tails had sterile uterine cavities. Thus there is some evidence to support the suggestion that also *in vivo* vaginal bacteria migrate along the mucus-covered tails of IUCDs.

The multifilamentous tail of the Dalkon shield has been shown to contain bacteria between the monofilaments within the outer sheath, (Tatum *et al*, 1975) the suggestion being that cracks in the outer sheath will release these bacteria into the uterine cavity. However, it is not clear whether such cracks are present in the outer sheath before removal of the IUCDs. Clearly this suggested mechanism for invasion of the uterus cannot account for the increased prevalence of pelvic infection in women using monofilamentous devices (Weström *et al*, 1976). The findings reported in our paper indicate that the use of any type of tail in an IUCD provides a potential route for infection of the uterine cavity. If confirmed by further clinical studies, it may be necessary to develop IUCDs which have no tails.

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A DOUBLE-BLIND CROSS-OVER STUDY OF THE EFFECT OF DOXYCYCLINE ON MYCOPLASMA INFECTION AND INFERTILITY

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Summary

Forty-two couples in an infertility clinic were examined for genital infection with *Mycoplasma hominis* or *Ureaplasma urealyticum* (T strains). In 21 couples one or other partner was found to be infected; no chlamydiae, trichomonads, or gonococci were isolated from any of these couples. All patients were then included in a double-blind crossover doxycycline/placebo study lasting 8 months. Pregnancy was achieved in one of the 21 couples who were free of infection. Of the 21 couples who were infected, one achieved a pregnancy while still infected, and 19 lost their infection on doxycycline, of whom four achieved a pregnancy. These four pregnancies all occurred among the 10 couples who received doxycycline first. One further pregnancy occurred after the trial was over. Since 5 of the 7 pregnancies occurred in the first 4 months of the trial, they were probably due to the psychological effect of treatment rather than the effect of the drug on infection.

IT has been suggested that genital infection with *Mycoplasma hominis* and *Ureaplasma urealyticum* (T strain) may cause infertility. Kundsin (1975) found that 15 out of 36 ureaplasma-positive women with unexplained infertility became pregnant within three months of treatment with declomycin. Gnarpe and Friberg (1972 and 1973*a*) found ureaplasmas in only 22 per cent of pregnant women but in 91 per cent of infertile women; 29 per cent of the latter group became pregnant after eradication of the infection with doxycycline. They also found ureaplasmas attached to the spermatozoa in semen from infertile males (Gnarpe and Friberg, 1973*b*). These findings have, however, not been confirmed by others (Louvois *et al*, 1974; Harrison *et al*, 1975; Matthews *et al*, 1975).

We therefore decided to do a double blind crossover trial of doxycycline and placebo on infertile couples with and without mycoplasma infections.

PATIENTS AND METHODS

Patients

Forty-two couples were included in this trial. All men had normal semen (more than 20 million sperm/ml) and all women were ovulating regularly (as judged by temperature charts, endometrial biopsies and/or urinary hormone studies) and had patent Fallopian tubes (as judged by hysterosalpingography and/or laparoscopy).

Specimens and treatment

Doxycycline or placebo (of identical appearance) was randomly allotted to each couple for the first three months of the trial. The tablets were given to both partners in three 10-day courses, during three successive menstrual cycles. One 100 mg tablet of doxycycline or one placebo tablet was given daily for the first two cycles and the daily dose was doubled on the third course. After an interval of one month without treatment, the couples who had not started a pregnancy then embarked on a second three months of treatment, those who had received placebo now being given doxycycline and *vice versa*. Cervical and vaginal swabs were taken for culture before the treatment started and at monthly intervals throughout the study. Specimens of semen were cultured and postcoital tests were each done thrice: once before treatment started, once between courses of treatment and once after the end of treatment.

Laboratory procedures

Postcoital tests were only judged as satisfactory when motile spermatozoa were present in large numbers.

Cervical swabs and semen samples were placed on horse serum mycoplasma broth and posted to the Mycoplasma Reference Laboratory. There they were inoculated onto manganese-urea-agar plates (based on the method of Shepard and Lunceford, 1976) and into urea broth with indicator, with and without lincomycin. The broth was subcultured onto manganese-urea-agar if it became alkaline. *M. hominis* was identified by disc growth inhibition and ureaplasmas by characteristic brown-staining colonies. Previous work did not suggest that there was likely to be any significant loss of infectivity on postage.

Further cervical swabs were then placed in Stuart's medium and were cultured for *Neisseria gonorrhoeae* and candida on modified chocolate agar (Cooper *et al*, 1950) and on trimethoprim-vancomycin-nystatin agar. Vaginal swabs were transported and cultured in trichomonas medium (Feinberg and Whittington, 1957) for *Trichomonas vaginalis*. Cervical swabs placed in chlyamydial transport medium (Richmond, 1974) were cultured for chlamydiae in cytochalasin-treated McCoy cells (Sompolinsky and Richmond, 1974). This last test was done only on preliminary specimens.

RESULTS

The two groups of patients were similar as regards age, duration of infertility, postcoital test results and the presence of mycoplasma infection. A larger proportion of those receiving doxycycline first had never been pregnant.

Mycoplasma infection

In the preliminary tests mycoplasmas were isolated from 20 of the 42 couples. *M. hominis* was found in 6 (14 per cent) female partners, 3 male partners and 7 (17 per cent) couples (one or both partners). *U. urealyticum* was isolated from 13 male partners, 12 female partners (29 per cent) and 17 (40 per cent) couples. Three women and one man were infected with both organisms. In addition, one man, whose wife remained uninfected, acquired a ureaplasma infection after the first three months' treatment. In 11 couples, mycoplasmas were isolated from only one partner. It is possible that more frequent sampling would have reduced the number of such couples.

Doxycycline proved effective in eradicating mycoplasma infection after the first ten day course in all but two couples infected with ureaplasmas; they were still infected at the end of the trial.

Other infections

No infections with *N. gonorrhoeae*, *T. vaginalis*, or *Chlamydia trachomatis* were found. Two women were infected with candida in the

TABLE I

Details of couples who achieved pregnancy

Doxycycline therapy	Mycoplasma infection		Time of conception in relation to:		Years of infertility	Postcoital test*
	Wife	Husband	Onset of trial	First negative specimen		
In first half of trial	*M. hominis* *U. urealyticum*	Negative	0	Before treatment started, when infection had disappeared spontaneously	2	U
	U. urealyticum	*U. urealyticum*	5 weeks	2 weeks after	3	S
	M. hominis	*M. hominis*	14 weeks	11 weeks after	2	S
	U. urealyticum	*U. urealyticum*	18 weeks	15 weeks after	2	S
	U. urealyticum	*U. urealyticum*	17 months	Infection not eradicated by doxycycline	5	U
In second half of trial	Negative	Negative	11 weeks†		6	V
	Negative	*U. urealyticum*	2 weeks	Still infected	2	S

* S = satisfactory; V = variable; U = unsatisfactory.

† Spontaneous abortion at 5½ weeks.

preliminary test, and eight became infected with these organisms during the course of treatment.

Pregnancies

Six couples achieved a pregnancy just before or during the study, and one couple achieved a pregnancy 10 months after the study. There were six live births and a spontaneous abortion (Table I). Table I also shows the status of the couples with regard to infection and its eradication.

The relation of pregnancy to infection is summarized in Table II. Though more pregnancies occurred in those who had lost their infection than in others, all four pregnancies occurred in the 10 couples who lost their infection after treatment in the first part of the trial and none among the 8 couples who lost their infection in the second part. Overall, five of the seven pregnancies occurred in the first four months of the trial.

TABLE II

Relation of pregnancy to infection with M. hominis and/or ureaplasma

Mycoplasma status at the time of conception	Number of couples	Number achieving pregnancy
Never infected during study	21	1
Infected	21	1*
Infection lost	19	4*

In addition a further couple, who had remained infected throughout the trial, achieved pregnancy after the trial was over, when it was not known whether they were infected or not.

* $p > 0{\cdot}05$; Bross's test (Bross, 1952).

Postcoital tests

The three serial tests in each couple showed quite marked variability but not such that the results could have been due to chance. Overall agreement between the result of the first and second test was 77 per cent. Unsatisfactory postcoital tests were found more often in couples who had never been infected (13 out of 21 couples) than in couples where one or both partners were infected with mycoplasmas (6 out of 21 couples). Unsatisfactory postcoital tests were also found more often when the male was not infected than when the male was infected with mycoplasmas. Eradication of the infection by doxycycline did not improve the result of the postcoital test; three unsatisfactory postcoital tests remained unsatisfactory, and 4 of the 13 couples with satisfactory postcoital tests developed unsatisfactory ones. This suggests that infection with mycoplasmas did not cause unsatisfactory postcoital test results.

The results of semen analyses were found to be so variable that the relation to mycoplasma infection could not be analysed.

Vaginal discharge

At the preliminary examination, vaginal discharge was noted in 9 (60 per cent) of the 15 women infected with mycoplasmas and in 3 (12 per cent) of the 25 women without infection with mycoplasma or candida.

DISCUSSION

In this study the infection rate of 14 per cent with *M. hominis* in infertile women and 17 per cent in infertile couples is similar to the infection rate of 14 per cent of infertile couples found by Louvois *et al* (1974) and 19 per cent of infertile women found by Love *et al* (1973). However, infection with ureaplasmas was lower in the present study (29 per cent of infertile women and 40 per cent of infertile couples) than found by Louvois *et al* (1974) (57 per cent of infertile couples), Matthews *et al* (1975) (56 per cent of infertile couples), or Gnarpe and Friberg (1972) (91 per cent of infertile women).

Since sexual exposure increases the likelihood of infection with mycoplasma, it is possible that the population studied here was not particularly sexually exposed, for organisms common among sexually exposed women, *N. gonorrhoeae*, *T. vaginalis*, or *Chlamydia trachomatis* were not found, although the techniques used for isolation were shown in the same laboratory to be sensitive, with an isolation rate for chlamydiae of 40 per cent in non-gonococcal urethritis.

On testing genital mycoplasmas *in vitro*, Spaepen *et al* (1976) found about 20 per cent resistant to doxycycline, and Evans and Taylor-Robinson (1978) found 10 per cent resistant to oxytetracycline. In the present study ureaplasmas from a comparable proportion of patients (2 out of 17) were not eradicated by doxycycline treatment. This was similar to the finding of Harrison *et al* (1975).

Although not statistically significant, the results of the present trial seemed to support the theory of Gnarpe and Friberg (1972) and Kundsin (1975) that the removal of mycoplasmas from the genital tract would result in pregnancy, for 4 of the 7 pregnancies occurred after mycoplasma infection had disappeared. However, the ritual of taking tablets by both husband and wife on certain days of the menstrual cycle might well exert a strong psychological effect. This idea is supported by the fact that all four pregnancies occurred in those who lost their infection and received doxycycline in the first half of the trial, and none occurred among the 8 couples who lost their infection and received doxycycline tablets in the second half. Our results thus support the findings of the double blind trial of Harrison *et al* (1975) that the removal of mycoplasmas did not increase the pregnancy rate. Neither Gnarpe and Friberg (1972) nor Kundsin (1975) had a control group of couples.

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ARTIFICIAL INSEMINATION IN DENMARK BY FROZEN DONOR SEMEN SUPPLIED FROM A CENTRAL BANK

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Summary

A central semen bank in Denmark supplied deep-frozen semen. Selection of donors, freezing technique, and practical details about transport of specimens are described. In one of the gynaecological departments supplied with frozen semen, 22 out of 30 patients who had artificial insemination became pregnant. The advantages and disadvantages of using frozen semen are discussed.

FOR the past 20 years frozen semen, stored in liquid nitrogen, has been used for artificial insemination by donor (AID) of normal women with success rates of 30 to 50 per cent (Behrman and Sawada, 1966; Lebech, 1972; Matheson *et al*, 1969; Perloff *et al*, 1964; Sawada and Ackerman, 1968). The success rate with fresh semen is said to be greater, variously reported as 55 to 78 per cent (Potter, 1958), about 70 per cent (Chong and Taymor, 1975; Friedman, 1977), 74 per cent (Jaszczak *et al*, 1974), and 40 per cent (Ilzuka *et al*, 1968).

In Denmark, AID using frozen semen has so far been used only to a limited extent (Lebech, 1972), but among the Danes there is now an increasing acceptance and demand for AID with waiting times of up to two years in clinics using fresh semen. The situation appears to be similar in Great Britain (Ledward *et al*, 1976).

We therefore feel that it is reasonable to describe our experience with the establishment of a central semen bank to serve gynaecological departments in the whole of Denmark.

METHODS

Donor panel

Since, as already mentioned, the conception rate seems to be lower with frozen than with fresh semen, we decided to accept only ejaculates with a volume above 2 ml, with a sperm concentration above 10^8/ml with not more than 30 per cent of morphologically abnormal sperms, and with excellent motility. In addition, we only accepted ejaculates in which 50 per cent of sperms remained mobile after trial freezing. Thus each ejaculate was frozen in portions of 0·5 ml and on the next day, one of the portions was thawed out and examined for sperm motility.

Of 200 donors (mainly students) about 50 had an initially acceptable semen volume and sperm count, but in only half of these was sperm motility satisfactory after a test freezing of semen. Once suitable donors had been identified, they were interviewed and examined by one of the authors to exclude mental and physical abnormality. Body build, hair and eye colour, and racial or other special characteristics were recorded. The donor's blood group was determined, a Wassermann reaction was done, and he was karyotyped. The donor panel ultimately consisted of about 20 men (or 10 per cent of those who had originally applied).

Freezing of semen

The semen was frozen immediately after liquefaction and always within 60 minutes of ejaculation. The method used was the one described by Perloff *et al* (1964). At room temperature, water-free, sterile glycerin was added, drop by drop, during magnetic stirring, up to a concentration of eight per cent. Thereafter, 0·5 ml volumes of the mixture were sucked into special plastic straws, care being taken to avoid bubbles of air, and the straws were sealed with polyvinyl alcohol powder. The straws were then laid horizontally onto a tray, in a container holding liquid nitrogen. The tray was placed at a level where the temperature has been found to be −30°C. After 15 minutes, the tray was moved down to a level where the temperature was −70°C and after a further 15 minutes the straws were immersed vertically in liquid nitrogen at −196°C.

Transport of semen

The Semen Bank was in central Copenhagen, and two methods of transport are used. Within Greater Copenhagen, specimens were ordered by telephone and transported in a taxi, being allowed to thaw *en route*, when the journey lasted under one hour. For longer journeys the semen was transported in liquid nitrogen. About 50 straws were sent to departments in other parts of the country capable of maintaining the level of liquid nitrogen in the containers and they were distributed from there. The clinics that received supplies from the central Semen Bank were spread throughout Denmark. Some specimens were also sent to Norway by air.

Insemination

Each gynaecological clinic decided on the indications for AID. We selected as a reference clinic the University Clinic in Århus, which is about 300 km from Copenhagen. In Århus, AID was used if the husband was thought, after three tests, to have azospermia, severe oligospermia, or sperms of greatly reduced motility. There were 30 women who had AID. All had kept basal body temperature charts for three cycles before treatment, had ovulation that could be predicted to within three days and luteal phases which lasted more than 10 days.

Insemination was usually done twice in each cycle, namely two days before and on the day of expected ovulation. Treatment was continued for a maximum of nine cycles. The insemination was done 20 to 30 minutes after the straw had been removed from the freezer. The straws were designed so that after the polyvinyl alcohol seal had been cut off, the seal at the other end was pushed through the straw by a stick, and the semen was thus injected into the mucus at the external cervical os. The patient lay on the examination couch for 20 minutes after the insemination. Care was taken to match donor and husband for hair and eye colour.

Of the 30 women treated, 22 became pregnant. The mean treatment period before conception was 3·4 cycles, while the mean period of insemination for all patients was 4·5 cycles. Two pregnancies ended in miscarriage, ten patients were delivered of normal infants, and ten are still pregnant.

Seven pregnancies occurred in the first treatment cycle, one in the second, five in the third, six in the fifth, and three in the seventh cycle.

DISCUSSION

Frozen semen has the advantage of being available at all times, because it can be stored. It makes it possible to match donor and husband much more accurately and to make allowance for blood group. Anonymity of the donor is more easily achieved by using frozen semen and in our system the gynaecologist who performs the insemination does not know the donor's identity.

When using frozen ejaculates from a semen bank, the result of bacteriological culture from the sperm is available before they are used. The importance of this factor is emphasized by the fact that *Neisseria gonorrhoeae* can survive deep-freezing to −196°C (Sherman and Rosenfeld, 1975). We have unfortunately been able to confirm this, since at the beginning, when the bank was being established, three women were infected with gonorrhoea after insemination with semen from the same donor. We now perform bacteriological control on every ejaculate. This safety precaution is not possible with the use of fresh semen for AID. The advantages of frozen semen seem to outweigh the possibly somewhat lower pregnancy rate, but it is possible that better results can be achieved by better freezing techniques and more reliable exclusion of donors whose sperm cannot tolerate freezing.

A risk of establishing a central semen bank in an area with a rather small population is that a few donors might father a great number of children. This would increase the possibility of in-breeding. We therefore have decided that a donor must be eliminated when his semen has produced a maximum of 15 pregnancies.

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SEXUAL DYSFUNCTION AND URINARY INCONTINENCE

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Summary

One hundred and three female patients attending an incontinence clinic gave information about their marital lives and sexual habits. Forty-eight women (46 per cent) maintained that their urinary disorder had adversely affected sexual relations. Women with bladder instability had a significantly higher incidence of sexual dysfunction than women with genuine stress incontinence.

THIS study searched to find out how often disorders of sexual function were present in women with urinary incontinence.

PATIENTS AND RESULTS

Female patients attending a urodynamic clinic for investigation were asked whether their urinary symptoms had interfered with their marital relations and sexual life in any way. An attempt was also made to identify any sexual or psychosexual problems that were not related to the urinary disorder.

One hundred and thirty four consecutive patients were interviewed. Of these, 9 were too old or infirm to give a proper history (mean age $\pm$ SD $= 72 \cdot 9 \pm 5 \cdot 8$ years), 21 were widowed, divorced or separated and one was an elderly spinster with no consort. One hundred and three patients aged between 20 and 68 (mean age $\pm$ SD $= 44 \cdot 7 \pm 7 \cdot 6$ years) were able to give details of their sexual life.

According to the results of urodynamic testing they were classified into groups as shown in Table I.

Forty-eight (46 per cent) of the 103 women admitted that their urinary symptoms had adversely affected their sexual life. In 36, sexual intercourse took place less frequently than before. In 12 it had ceased altogether.

The reasons for the reduced frequency of sexual intercourse were superficial or deep dyspareunia (most common), wetness at night and the need for the protection of a towel all the time, leakage of urine during coitus, decreased libido, depression or embarrassment, marital discord and the need for separate beds (least common).

Women with bladder instability appeared to suffer more from dyspareunia than women in other groups. These women also experienced bed-wetting before and during coitus. Three patients with dyspareunia had had previous vaginal surgery, and their dyspareunia was partly due to scarring of the vagina.

There was no significant difference between patients with genuine stress incontinence and those with an unstable bladder with regard to age or parity. However, unsatisfactory sexual relations due to incontinence were present in 28 (68·3 per cent) out of 41 patients with bladder instability, compared with 7 (20·6 per cent) out of 34 patients with genuine stress incontinence ($\chi^2 = 15 \cdot 132$, $p < 0 \cdot 001$).

DISCUSSION

More than two million people in Britain are troubled by urinary incontinence (Leading Article, 1978). Its effect on older people has

TABLE I

Urodynamic diagnosis and sexual function in the 103 women in the study

Diagnosis	Number in group	Number with sexual dysfunction due to urinary disorder	Number with sexual dysfunction due to other reasons	Number with no sexual dysfunction
Genuine stress incontinence	34	7	10 (Husband: Stroke 1 Bronchitis 2 Lack of libido 1 Wife: Prolapse 2 Bronchitis 1 Lack of libido 3)	17
Unstable bladder	41	28	3 (Husband: Stroke 1 Carcinoma 1 Wife: Prolapse 1)	10
Stress incontinence and unstable bladder	10	5	1 (Husband: Prostatectomy)	4
Urethritis/trigonitis	11	6	1 (Husband: Lack of libido)	4
No abnormality detected	7	2	1 (Wife: Retroverted uterus)	4
Totals	103	48	16	39

received attention (Willington, 1969; Milne *et al*, 1972), but the problems which face younger women have not been fully assessed.

This study has shown that women with urinary incontinence, particularly that due to bladder instability, commonly have associated sexual dysfunction.

The aetiology of the unstable bladder is unclear. Occasionally there is an underlying neurological fault, but usually no abnormality can be detected (Hald, 1975). The urgency incontinence commonly present in women with detrusor instability has been described as a psychosomatic disorder (Frewen, 1970; Jeffcoate and Francis, 1966). This hypothesis would be supported if psychological or psychosexual symptoms appeared before the onset of detrusor incontinence. Most of the women interviewed in this study had been incontinent for many years, and one could not be sure which disorder came first. However, all of the women maintained that their urinary incontinence was the cause and not the effect of their disturbed sexual function. Furthermore, in those patients whose sexual life had been affected by other conditions (Table I), the urodynamic diagnosis was more commonly genuine stress incontinence than unstable bladder. These observations would suggest that lack of sexual fulfilment is not a major cause of bladder instability.

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SPONTANEOUS PREGNANCY IN A PATIENT WITH HYPERGONADOTROPHIC OVARIAN FAILURE

BY

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Summary

A 27-year-old woman was referred to the gynaecological endocrine clinic because of infertility and secondary amenorrhoea. Serum luteinizing hormone and follicle stimulating hormone levels were raised and oestradiol concentrations were subnormal. In spite of these findings, spontaneous resumption of menstrual cycles associated with a fall of gonadotrophin concentrations was followed by a normal pregnancy.

ABOUT 10 per cent of patients referred to a gynaecological endocrine clinic because of amenorrhoea have primary ovarian failure (Jacobs *et al*, 1977). This condition is caused by exhaustion of the ovarian store of germ cells. The lack of oocytes causes sterility and the lack of follicles causes a fall in the rate of secretion of oestradiol and a consequent rise of gonadotrophin levels. The condition is generally considered to be irreversible.

We report here details of a young woman in whom secondary amenorrhoea associated with the typical endocrine findings of primary ovarian failure was followed by resumption of menstrual cycles and a normal pregnancy.

CASE REPORT

In November 1976, a 27-year-old woman was referred to the gynaecological endocrine clinic because of secondary amenorrhoea and infertility. Her menarche had been at the age of 15 and she had subsequently had an irregular 21 to 35 day menstrual cycle until she began oral contraceptive therapy at the age of 23. Two years later she discontinued oral contraceptives and after three months without a period, a withdrawal bleed was induced by a course of oral progestogens. She had a spontaneous period one month later and then became pregnant. The pregnancy aborted spontaneously at three months gestation. It was followed by six months of amenorrhoea and then, after four spontaneous periods, a further seven months of amenorrhoea, at the end of which time she was referred for investigation.

There was no history of recent systemic illness, weight change, drug therapy or galactorrhoea. She had not suffered from hot flushes. General examination revealed a healthy woman (height 155 cm, weight 49·4 kg) with normal secondary sexual characteristics and no evidence of masculinization. Examination of the lower genital tract revealed a poorly oestrogenized vagina. The pelvic organs were otherwise apparently normal.

The serum follicle stimulating hormone (FSH) concentration was 700 μg/l (in our laboratory the normal premenopausal range is 12·5 to 50 μg/l (MRC 68/39)) and the serum luteinizing hormone (LH) concentration was

22 μg/l (normal premenopausal range is 0·5 to 2·0 μg/l (MRC 68/40)). The serum oestradiol was 17 nmol/l (normal range for mid-follicular phase in premenopausal women is 21 to 42 nmol/l). The serum prolactin concentration was 4 μg/l (normal range is 3·0 to 15 μg/l (VLS No. 3)) and the serum thyroxine was 127 nmol/l (normal range is 70 to 160 nmol/l). An X-ray of the pituitary fossa was normal. Cytological examination of a cervical smear showed an atrophic pattern. The karyotype of cultured peripheral leucocytes was 46XX.

Further investigation followed the protocol of Jacobs *et al* (1975). The patient failed to develop withdrawal bleeding after treatment with oral medroxyprogesterone acetate (5 mg a day for five days) but probably responded to clomiphene (100 mg a day for five days). Thus on days 15 and 18 after the clomiphene was started, serum oestradiol levels had risen to 52 and 90 nmol/l and serum progesterone concentrations were 4 and 16·5 nmol/l respectively and the patient had vaginal bleeding on the 33rd day. She received no further drugs but menses returned and the cycle length diminished (41, 37, 31 and 30 days) (Fig. 1). During the fourth cycle there was presumptive evidence of ovulation, with a sustained shift of the basal body temperature. At the beginning of this cycle serum gonadotrophin concentrations, though lower than at presentation, were still in the postmenopausal range. During the next cycle the patient conceived. During the eight months preceding conception, serum concentrations of gonadotrophins, pro-

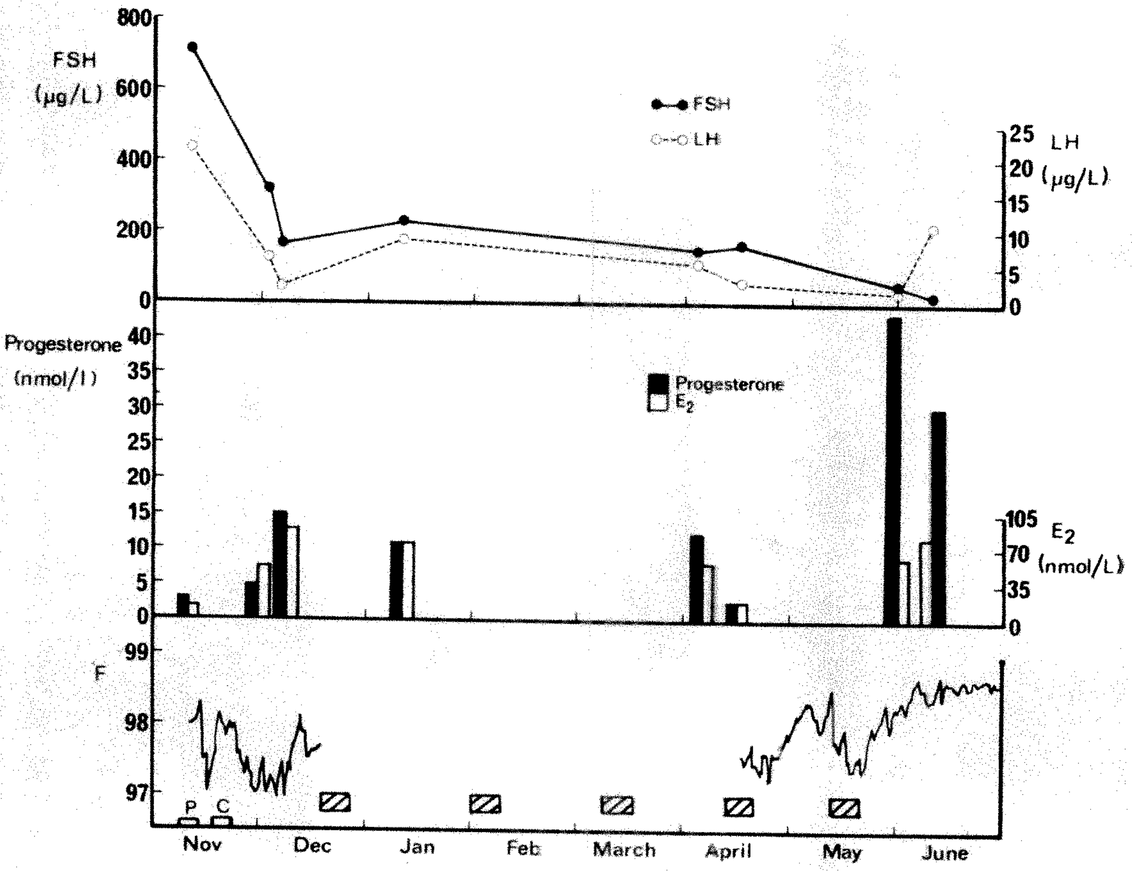

FIG. 1

Serum FSH, LH, progesterone and oestradiol measurements and basal body temperature in the seven months preceding pregnancy (P = treatment with medroxy progesterone acetate; C = treatment with clomiphene).

gesterone, and oestradiol were measured regularly and the results are shown in Figure 1. Pregnancy was uneventful and the patient was delivered of a normal live male infant at 39 weeks gestation (birthweight 2·75 kg) in February 1978. Lactation was normal and she breast fed for 3 months.

At 6 weeks post partum the serum gonadotrophin concentrations were again in the postmenopausal range (LH = 4·3 μg/l, FSH = 290 μg/l). Her first period was 8 months post partum. Until two weeks before this period the patient noted dyspareunia and loss of libido. The serum gonadotrophin concentrations after this episode of vaginal bleeding were in the postmenopausal range. Further investigation by laparoscopy and ovarian biopsy was offered but the patient declined.

Discussion

The association of amenorrhoea with gonadotrophin levels raised into the menopausal range is found in three conditions—primary ovarian failure, the 'resistant ovary' syndrome and congenital 17 alpha-hydroxylase deficiency. In the last condition the enzyme deficiency results, inter alia, in a failure to secrete oestradiol (Biglieri *et al*, 1966) but the ovaries contain oocytes and follicles, albeit poorly developed (Mallin, 1969). In the 'resistant ovary' syndrome, oocytes and follicles are also present though their histological appearance is abnormal (Van Campenhout *et al*, 1972; Dewhurst *et al*, 1975). Nevertheless, despite high endogenous gonadotrophin concentrations, and resistance to treatment with gonadotrophins (Van Campenhout *et al*, 1972), oestrogen production is not impaired (Dewhurst *et al*, 1975). Primary ovarian failure is characterized by subnormal oestradiol secretion, high gonadotrophin concentrations and complete or nearly complete depletion of oocytes and ovarian follicles (Goldenberg *et al*, 1973).

In the patient we describe, 17 alpha-hydroxylase deficiency was readily excluded by the history and the clinical and endocrine findings. The 'resistant ovary' was considered unlikely because of the subnormal oestrogen production at presentation, as indicated by the failure to bleed after progestogen challenge and the low serum oestradiol concentration. However, it is accepted that diagnosis of this condition can only be excluded with certainty by direct histological examination of ovarian tissue.

There are several causes of primary ovarian failure (Jacobs and Murray, 1976), most of which are considered to be irreversible. However, resumption of ovulatory cycles, in one case followed by a pregnancy, has been recorded in women developing amenorrhoea, high gonadotrophin concentrations and low oestradiol levels after ovarian irradiation (Thomas *et al*, 1976). This pattern of events may also follow cytotoxic chemotherapy (Thomas and Jacobs, in preparation). In the patient we describe, these conditions were excluded by the history. A viral oophoritis causing temporary damage, as with mumps (Morrison *et al*, 1975), is a theoretical explanation but there was no history of a systemic illness preceding the development of amenorrhoea.

In perimenopausal women, Sherman and Korenman (1975) have described the development of irregular menstrual cycles associated with elevated concentrations of one or both gonadotrophins. In some of the patients described by these workers, ovulation could be inferred from measurements of serum progesterone concentrations. It is possible that the patient we describe represents an example of a 'premature perimenopause', the temporary ovarian resistance to gonadotrophin (indicated by the high gonadotrophin concentrations with low plasma oestradiol levels) being analogous to that which occurs in the normal menopausal transitional phase of reproductive life (Sherman and Korenman, 1975). In the absence of histological confirmation, this diagnosis must, however, remain speculative, but it is supported by the return of elevated gonadotrophin concentrations in the samples obtained post partum.

Patients with similar details have been described by Polansky and de Papp (1976) and Shangold *et al* (1977). In both patients ovarian biopsies, obtained at laparoscopy, showed a total absence of ova. Dewhurst *et al* (1975) have shown, however, that occasionally follicles may be present only deep in the ovarian parenchyma, a finding that probably explains the biopsy appearance described by Polansky and de Papp (1976). The only other occurrence of a spontaneous pregnancy in a patient with hypergonadotrophic amenorrhoea was reported by Shapiro and Rubin (1977) but ovarian histology was not obtained.

Thus, 'primary ovarian failure' may on occasion be a reversible condition. In young women with amenorrhoea and high gonadotrophin concentrations, particularly those requesting a definite prognosis about future fertility, laparoscopy is indicated. If the gonads are streaks or obviously atrophic, no further investigation is called for. However, if the ovaries are of normal size or enlarged, then a 'mini-laparotomy' should be performed and an ovarian biopsy taken to obtain, as far as possible, tissue representative of the histological structure of the whole ovary.

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NOTE

Since this report was submitted, the patient has had one more spontaneous menstrual period. She conceived two weeks later, and is now in the second trimester of pregnancy.

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ISOLATED ARTERITIS OF THE CERVIX UTERI

BY

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Summary

Four patients with arteritis apparently isolated to the cervix uteri are presented and the clinical and pathological significance of this condition is discussed.

THERE have been a number of reports of arteritis, with histological features of polyarteritis nodosa, but apparently confined to one organ (Plaut, 1951; Bohrod and Bodon, 1970; Feasby *et al*, 1975; Ansell *et al*, 1974). We would like to report four patients with arteritis of the cervix uteri. They were discovered by routine histopathological examination over a period of three years.

CASE REPORTS

Patient 1

The patient, aged 18 and para 0+0, presented as an emergency with a history of fainting and lower abdominal pain. One week previously she had consulted her general practitioner because of lower abdominal pain which was diagnosed as salpingitis and treated by ampicillin. At examination under anaesthesia and laparoscopy to exclude ectopic pregnancy, the pelvic organs showed mild congestion consistent with resolving pelvic inflammatory disease. During examination of the cervix an ulcer of the anterior cervical lip was noted and biopsied. There was no past medical history. The patient had no further treatment and made an uneventful recovery. Two cervical smears taken after operation showed no malignant cells.

Histological examination showed necrotic cervical tissue and vasculitis of the small arteries with fibrinoid necrosis of the muscular walls and a surrounding mixed acute and chronic inflammatory cell infiltrate (Fig. 1).

Six weeks after laparoscopy the cervix was treated with the cryoprobe and the patient has subsequently remained well.

Patient 2

The patient, aged 62 years and para 2+1, had reached the menopause 21 years previously. She was admitted to hospital with a history of sudden profuse postcoital bleeding. The patient had a history of duodenal ulcer and hypertension which was being treated with propanolol, methyldopa and diuretics. She had previously been on bethanidine.

The blood pressure was 90/60 mm Hg and a loud pansystolic murmur was heard. There was blood clot in the vagina and at examination under anaesthesia the cervix appeared to be infiltrated by a hard growth. Biopsies of the cervix were taken on two separate occasions.

A sedimentation rate had previously been 23 mm/hour. An electrocardiograph showed widespread ischaemic changes and old inferior infarction while chest X-Ray showed mitral valve calcification and an enlarged right atrium.

Histological examination of the first biopsy of the cervix showed many inflammatory cells in the stroma associated with an arteritis

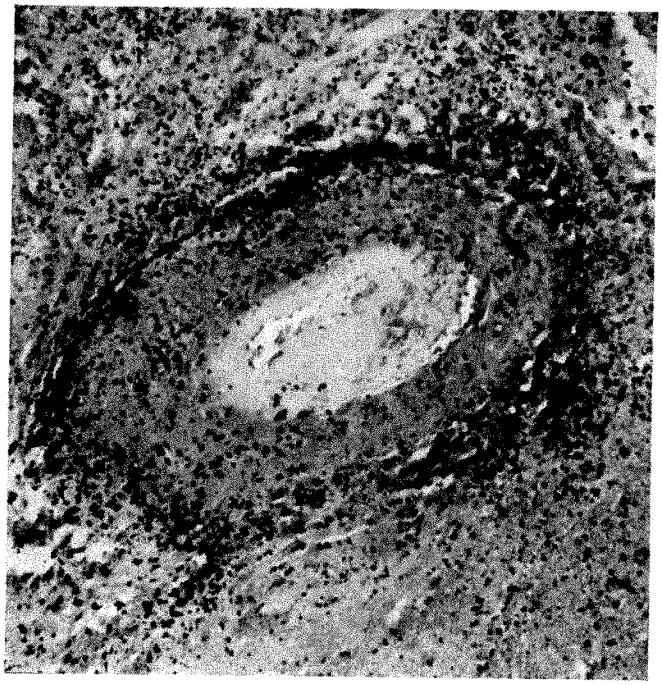

FIG. 1

Patient 1. Fibrinoid necrosis and inflammatory cell infiltration of a small artery with surrounding tissue necrosis (haematoxylin and eosin ×112).

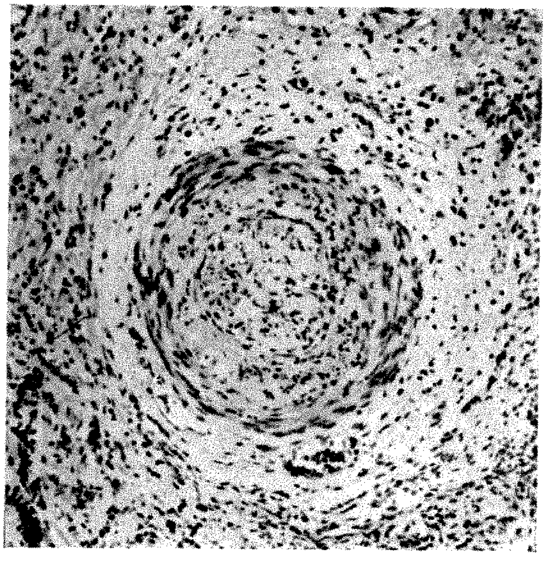

FIG. 2

Patient 2. Endarteritis obliterans with surrounding inflammatory cell infiltrate (haematoxylin and eosin ×112).

consisting of thickening of vessel walls, endarteritis obliterans and an infiltration of inflammatory cells (Fig. 2). The second biopsy of the cervix showed only mild inflammation and no arteritis. There was no evidence of carcinoma.

The patient has been seen at regular intervals and there has been no recurrence of symptoms. Direct examination of the cervix has shown no abnormality and cervical smears have been consistently negative.

Patient 3

This patient, aged 78 years and para 1+0, presented with a prolapse and vaginal bleeding. She had had rheumatic fever as a child and a negative investigation for an episode of diarrhoea two years previously. She was being treated with methyldopa and diuretics for hypertension.

The blood pressure was 130/85 mm Hg and there was a systolic ejection murmur. Vaginal examination showed the cervix to be congested.

The ESR was 26 mm/hour. An ECG showed old inferior infarction and lateral wall ischaemia. A chest X-Ray showed left ventricular enlargement, calcification of the aortic valve and unfolding of the aorta. The cervical smear showed an atrophic pattern with no malignant cells. A vaginal hysterectomy and anterior and posterior repair were done and a postoperative bacteroides septicaemia responded to a combination of antimicrobial drugs.

Naked eye examination of the excised uterus showed a large subendometrial fibroid and a thickened, deformed cervix. Histology confirmed the presence of benign leiomyomata and there was atrophy and polypoidal degeneration of the endometrium. There was hyperkeratosis and squamous metaplasia of the endocervix and an isolated arteritis affecting two small vessels deep within the wall of the cervix. In one of these vessels there was almost complete destruction of the vessel wall associated with infiltrating chronic inflammatory cells (Fig. 3).

Six weeks after operation the patient was readmitted to hospital with anaemia, congestive cardiac failure and a pruritic rash on the legs which was thought to be a reaction to antimicrobial drugs. The rash subsided spontaneously and the patient's cardiac condition improved.

Patient 4

The patient, aged 41 and para 3+0, presented with continuous vaginal bleeding of six weeks' duration. Curettage of the uterus 18 months previously had failed to relieve menorrhagia which had been present for two years. There was a soft systolic ejection murmur and the blood pressure was 130/70 mm Hg. No abnormality was noted on vaginal examination. A full blood count and ESR were normal. The patient was given 5 mg norethisterone three times daily whilst awaiting admission to hospital for hysterectomy and bilateral salpingo-oophorectomy.

The excised uterus showed no abnormalities and both ovaries contained small follicular cysts. Histologically, the endometrium was in the proliferative phase and there was a small intramural fibroid. The cervix showed mild cervicitis with mucoid retention cysts and a marked arteritis affecting some vessels deep in the wall of the cervix. There was fibrinoid necrosis of part or all of the vessel wall and a surrounding chronic inflammatory cell infiltrate of lymphocytes and plasma cells (Figs. 4 and 5).

Immunoperoxidase studies showed that the areas of 'fibrinoid necrosis' which stained red with Martius Scarlet Blue also showed positive staining with anti-fibrinogen antiserum. There

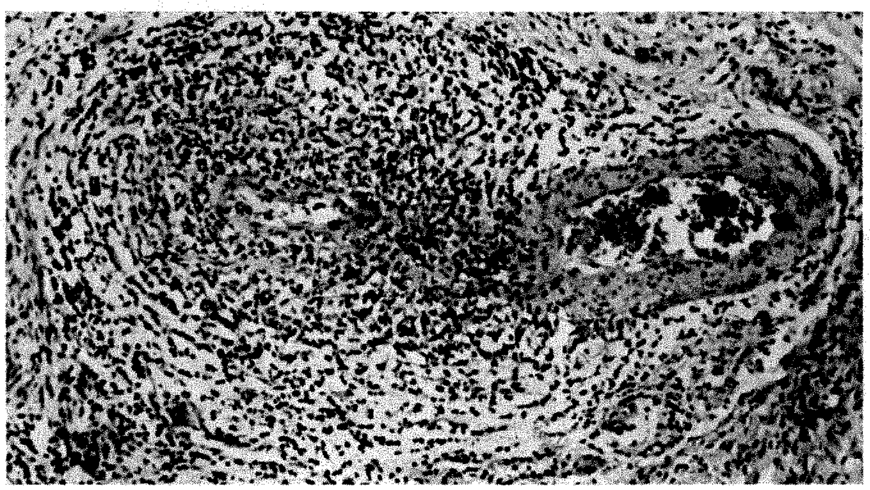

FIG. 3

Patient 3. Complete destruction of the wall of a small artery associated with an inflammatory cell infiltrate (haematoxylin and eosin ×112).

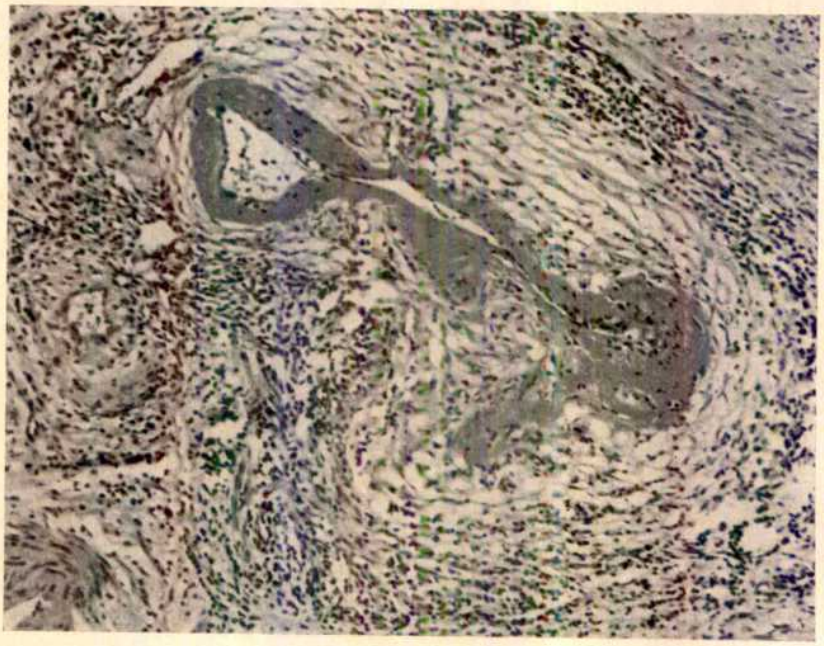

FIG. 4

Patient 4. Complete fibrinoid necrosis of arterial wall with surrounding inflammatory cell infiltrate (haematoxylin and eosin ×112).

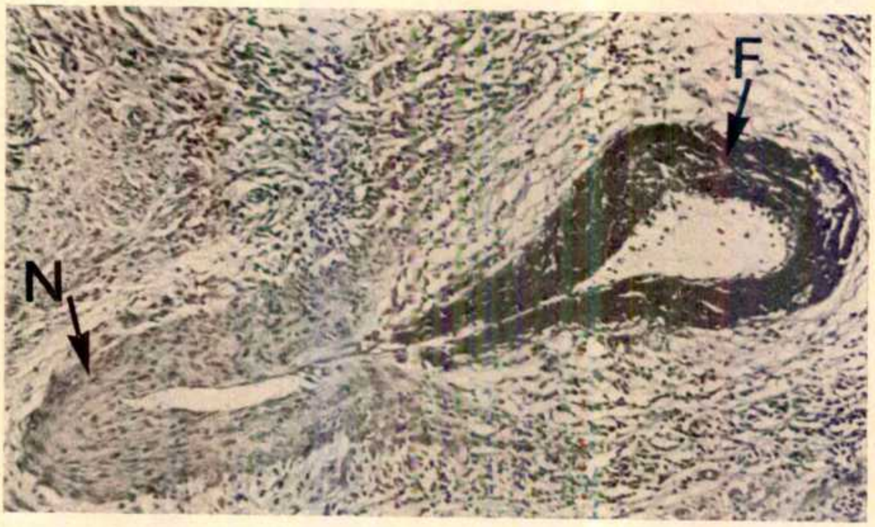

FIG. 5

Patient 4. Arteritis affecting one half of a vessel wall with fibrinoid necrosis (F) and inflammation but sparing the left side which appears normal (N) (Martius Scarlet Blue ×112).

was however no such staining with antisera to IgG, IgA, IgM, K and L chains and complement (C_3) although there was a faint staining with all of these of the intraluminal serum in both normal and abnormal vessels and a stronger staining of all except complement in the cytoplasm of the individual lymphoid cells. Thus we were unable to demonstrate any evidence of an antigen/antibody reaction in the necrosed wall.

Six weeks after the operation the patient had recovered fully.

DISCUSSION

The arterial changes we found were similar to those found in polyarteritis nodosa but none of our patients showed evidence of generalized disease. We thus appear to be dealing with 'isolated' or 'asymptomatic' arteritis. Isolated arteritis has been described in the appendix (Plaut, 1951), the gall bladder (Bohrod and Bodon, 1970), the spinal cord (Feasby *et al*, 1975) and the cervix uteri (Ansell *et al*, 1974). Ansell *et al* (1974) described 10 patients; their ages ranged from 34 to 52 years, and the cervical arteritis was an incidental finding apparently unrelated to their gynaecological symptoms.

The aetiology and pathogenesis of isolated arteritis remain obscure but it is of interest that two of our patients had evidence of ischaemic heart disease, cardiac valve disease and hypertension and that one of the patients described by Ansell *et al* (1974) also had hypertension.

The Committee on Safety of Medicines has on its records the reports of two patients with arteritis in which methyldopa may have played a part (Greenberg, G. personal communication). One was a 69-year-old woman who had been treated with methyldopa, cyclopenthiazide, potassium chloride and digoxin for 14 months and developed temporal arteritis. The other was a 50-year-old woman who had been on methyldopa for two years when she developed an unusual arteritis affecting small vessels in the feet. Treatment with methyldopa and bendrofluazide was continued for a further two years when a diagnosis of Buerger's disease was made. It is of course quite possible that the vascular lesions in question could have been purely coincidental and not related to drug therapy.

Arteritis may be a manifestation of local infection, but if that is the case it is strange that the vessels seem to be the main site of the inflammation. The absence of immunoglobulins in the vessel walls in Patient 4 does not preclude an immunological reaction (perhaps to a local infection) since it is often impossible to demonstrate immunoglobulins in patients in whom vascular damage is known to be associated with circulating immune complexes (Denman, A. M., personal communication) and if the lesions are not in the acute stage the protein complexes in the vessel walls may be degraded by neutrophils (Gell *et al*, 1975). It is also possible that vasculitis may be due to viruses and mycoplasmas without immunological mediation (Christian and Sergent, 1976).

Vasquez and Dixon (1958) found evidence of two types of fibrinoid immunohistochemical reactions. In systemic lupus erythematosus, rheumatic fever and experimental serum sickness the fibrinoid stained preferentially for immunoglobulin whereas in thrombotic thrombocytopenic purpura, the Schwartzman phenomenon and adrenocortical necrosis of abruptio placentae it stained preferentially for fibrin/fibrinogen. Patient No. 4 would appear to fall into the latter group although there was no evidence of a generalized disease process.

Isolated arteritis of the cervix uteri may be commoner than is generally realised. It may be asymptomatic or associated with tissue necrosis and bleeding when it may mimic a cervical carcinoma. Histologically, the lesion resembles isolated arteritis in other organs and also the lesions seen in generalized arteritis. Isolated arteritis appears to be unrelated to classical polyarteritis nodosa and the aetiology is unknown.

ACKNOWLEDGEMENTS

We thank Mr H. Gordon and Mr A. Fisher under whose care the patients were admitted and Dr G. Slavin for advice on histopathology. Mrs G. Sowter performed the immunoperoxidase reactions and Mr. J. B. Clark the photography.

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MALIGNANT CHANGE IN BENIGN CYSTIC TERATOMA OF THE OVARY

BY

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Summary

In a clinico-pathological study of malignant disease of the ovary in the former North East Metropolitan Region, malignant transformation of benign cystic teratoma has emerged as the commonest malignancy of 'germ cell' origin. The clinical and pathological details of 12 patients are reported.

BENIGN cystic teratoma is probably the commonest ovarian tumour, the incidence reported being of the order of 20 per cent of specimens examined (Fox and Langley, 1976). It is considered to be of germ cell origin and frequently contains derivatives of all three germ layers. The most common complication is torsion (approximately 10 per cent; Caruso *et al*, 1971).

Endocrine effects and an unusual autoimmune haemolytic anaemia have been reported (Bernstein *et al*, 1974). Rupture is rarer (approximately 1 per cent; Peterson *et al*, 1955). Malignant change was found by Peterson (1957) in 2 per cent of specimens examined. Malignancy had extended beyond the ovary in 64 per cent of their patients and the five year survival for such patients was only 6 per cent.

We now draw attention to the incidence of this type of malignancy among primary ovarian tumours and discuss special problems of diagnosis and management.

METHODS

The data reported are derived from a collaborative prospective study set up under the joint auspices of the Association of Obstetricians and Gynaecologists and the Regional Histopathologists Group of the North East Thames Region (Curling and Hudson, 1975). Patients with ovarian cancer presenting for primary treatment in the hospitals of the former North East Metropolitan Region are entered in a Tumour Registry with a standard presentation protocol. The histological material is examined by the investigators (O.M.C. and C.N.H.) without prior reference to the report by the local pathologists. Discrepancies, difficult and interesting tumours are referred to a special group of interested pathologists (see Acknowledgements).

The patients reported are from among 686 entered in the study; 600 of these were designated as primary tumours of the ovary, the remainder being considered as secondary tumours or of uncertain origin. One of the

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TABLE I

Tumours of germ cell origin (N.E. Thames Series)

(a)	Disgerminoma	5
(b)	Endodermal sinus tumour	4
(c)	Embryonic carcinoma (combination a and b)	1
(d)	Solid teratoma (doubtful malignancy)	4*
(e)	Malignant change in cystic teratoma	11
(f)	Monophyletic tumour (doubtful malignancy)	2 (struma/carcinoid)

There have been four patients with malignant melanoma involving the ovary. In two the primary site was unknown, but there was no evidence of a pre-existing cystic teratoma.

* One patient had a benign cystic teratoma in the opposite ovary.

secondary cancers showed extrinsic involvement of a benign bystic teratoma.

A summary of the primary malignant tumours considered as being of germ cell origin is shown in Table I. This demonstrates a preponderance of those considered to be malignant change in a presumably previously benign cystic teratoma. Individual case histories are summarized in Table II.

DISCUSSION

Although malignant change in dermoid cysts has been recognised for many years (Counsellor and Wellbrock, 1934), reports have tended to indicate that the condition is a clinical and pathological oddity (Alznauer, 1953). It is obvious, however, that this is the most serious complication of benign cystic teratoma and as such should be more carefully evaluated.

The age range of the patients reported here is 33 to 74 years (peak incidence 55 to 60 years). This compares with a reported peak incidence in the fifth and sixth decades (Kelly and Scully, 1961). Malkasian *et al* (1967) found 30 per cent of 612 cystic teratomata occurring after the age of 50 and in this age group the risk of malignant change is doubled.

The clinical diagnosis of malignancy at operation is easy when metastasis has occurred, except that the appearance of neglected rupture of a benign cyst may mimic malignancy (Quer *et al*, 1951). The detection of malignant change in an unruptured cyst is less easy and caution should be observed when handling an adherent 'dermoid' cyst in a postmenopausal patient.

The histological diagnosis is relatively simple in most cases. There are, however, some recognised 'pitfalls' (Matz, 1961). Malignant change in a benign cystic teratoma needs to be distinguished from solid teratoma as many examples of the latter will contain small cysts. The age distribution is different, and the presence of benign squamous epithelium in part of the cyst wall strongly indicates the former diagnosis. The distinction from mixed mesodermal tumour may also be difficult (there are five patients with mixed mesodermal tumour among those studied here); the diagnosis of teratoma depends on detection of ectodermal or endodermal elements, which is not always easy if the malignant change is sarcomatous and exuberant (Burgess and Shutter, 1954). The situation is further occasionally confused because the epithelial element may rarely show squamous metaplasia (Fox and Langley, 1976). The presence of rhabdomyoblasts must however be almost pathognomonic of mixed mesodermal tumour.

Finally, the prediction of malignant behaviour in a cystic teratoma may be difficult if neural elements predominate. A cautious prognosis should always be given if peritoneal implants are present even of seemingly benign glial or thyroid tissue (gliosis, strumatosis).

The natural history of the disease suggests that initial spread is likely to be local extension before lymphatic or transcoelomic dissemination occurs, although this may not be the case with sarcomatous change (Climie and Heath, 1968). Such cases would appear to be suitable for wide local excision using the retroperitoneal radical oöphorectomy technique (Hudson, 1968). Carcinoma of the endometrium has been reported in association with benign 'dermoid' cyst (Caruso *et al*, 1971) but one suspects that it was the 'dermoid' that was the chance finding.

Although hysterectomy may not be mandatory, there can be no grounds for ovarian conservation in a postmenopausal patient.

Neither the earlier reports nor this small series lend much support to the use of radiotherapy for these tumours. Chemotherapy has

TABLE II

Malignant change in dermoid cysts
(N.E. Thames Series)

No.	Age	Stage	Treatment	Outcome	Pathology
562	57	1A	Hyst. B.S.O.	Alive and well; 3 years	Squamous cell
897	56	1A (nearly perforated)	Hyst. B.S.O. + DXR	Died; 14 months	Squamous cell
528	33	1A	Unilateral oophorectomy	Rapid recurrence, unresponsive chemotherapy; died; 9 months	Undifferentiated
570	46	1A	B.S.O. Hyst.	Alive and well; 3 years	Squamous cell
95	73	1A	Hyst. B.S.O.	Lost to follow up	Poorly differentiated squamous cell
446	67	2B	Incomplete surgery; Hartmans operation + DXR	Died; 4 months	Squamous cell
488	51	3	Biopsy	Operative death	Squamous cell metastasis; primary tumour examined at op only
25	55	1A	Hyst. B.S.O.	Alive and well; 7 years	Squamous cell
174	74	1A	Unilateral oophorectomy, incomplete	Died; 3 months	Adenocarcinoma believed sweat gland origin
454	65	2B	B.S.O.	Died; 7 months	Squamous cell + sarcomatous areas
712	56	3	Bilateral oophorectomy + small bowel resection. Incomplete	Treated bleomycin; Died; 3 months	Squamous cell
895	70	1A	Hyst. B.S.O.	Alive and well; 3 months	Squamous cell

Hyst. = Hysterectomy; B.S.O. = Bilateral salpingo oophorectomy.

certainly not been satisfactorily evaluated, single alkylating agent therapy having proved disappointing on the occasions when used. One may speculate that combination therapy should be used, and the known activity of bleomycin against both squamous cell cancer and certain types of teratoma would suggest that any regimen should contain this agent, although single agent activity was not demonstrated in the one patient treated with a single agent in this series.

ACKNOWLEDGEMENTS

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MIXED MESENCHYMAL SARCOMA OF THE BROAD LIGAMENT
CASE REPORT

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Summary

A 55-year-old patient who presented with a pelvic mass was found to have a mixed mesenchymal sarcoma of the broad ligament. The histogenesis and diagnostic criteria of this rare tumour are discussed.

PRIMARY sarcomas of the broad ligament are exceedingly rare (Novak and Woodruff, 1974) and most malignant tumours at this site are regarded as having spread from a uterine or ovarian primary (Haines and Taylor, 1975).

In presenting this case report we wish to adduce evidence that a mixed mesenchymal sarcoma can arise in the broad ligament and discuss the problems in diagnosis and management of a patient with a tumour of this type.

CASE REPORT

A 55-year-old patient presented with a history of postmenopausal vaginal bleeding and lower abdominal pain for one month. On examination she appeared pale and ill. She had a very tender pelvic mass which was thought to be an ovarian cyst or a pedunculated fibroid which had undergone torsion or degeneration. The haemoglobin was 11·3 g/dl, the white cell count 8500/mm^3 with a normal differential count. The plasma urea and electrolytes were normal. A laparotomy was done on the day of admission. The peritoneal cavity contained free blood and a firm tumour was found attached to the right pelvic wall, the right Fallopian tube, the right broad ligament and uterus. Because there was uncertainty about the diagnosis it was decided not to undertake a pelvic clearance at this time but to perform a more limited operation and await histopathological opinion. A right salpingectomy was performed and most of the tumour was removed. The raw area was packed with oxycel and the abdomen closed. Dilatation and curettage produced scanty fragments of glandular epithelium with no evidence of an intrauterine neoplasm. The operation specimen consisted of pieces of friable white and haemorrhagic tissue together forming a pile 10 × 8 × 5 cm and a 3 cm length of Fallopian tube with some firm white tissue at its distal end and in the attached mesovarium. Microscopy showed a mixture of polygonal and spindle cells. The former showed solid, trabecular, tubular or tubulopapillary arrangements while the latter lay in bundles (Fig. 1). In addition, there were whorls of spindle and stellate cells in a loose matrix, elongated strap and tennis racquet shaped cells with large nuclei and eosinophilic cytoplasm (Fig. 2) containing indistinct striations; foci of chondroblastic differentiation were also apparent (Fig. 3). Many mitotic figures were present. The tumour obliterated the distal tubal architecture and extended into the attached mesovarium.

Postoperatively, the patient had severe bronchopneumonia. She was eventually considered fit for further surgery after 30 days and had a total hysterectomy and bilateral salpingo-oophorectomy. Attached to the right posterior surface of the uterus there was a rubbery nodule 1 × 1 × 0·5 cm. Histological examination of this nodule showed fibrous tissue containing aggregates of malignant polygonal cells showing tubular differentiation. The attached remnant of the right broad ligament contained a fairly well circumscribed nodule of pleomorphic polygonal cells lying in a spindle cell stroma. Similar aggregates of cells were present on the surface of the right ovary but there was no evidence of deep ovarian involvement. The right Fallopian tube showed surface aggregates of pleomorphic polygonal cells with some tubular differentiation

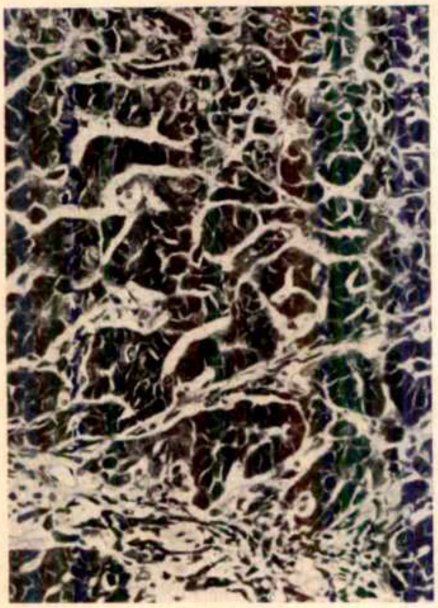

FIG. 1

Tumour showing pronounced tubular differentiation (haematoxylin and eosin ×33).

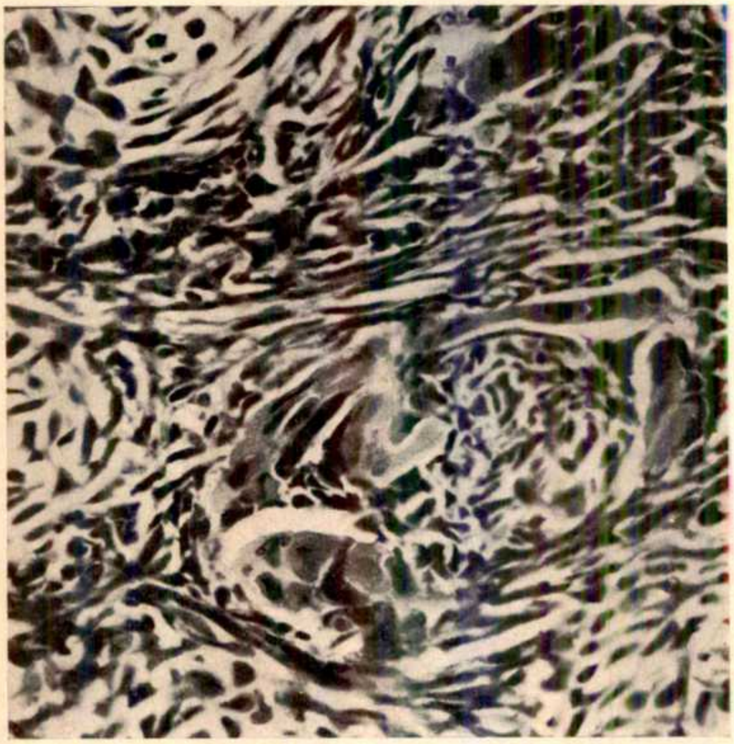

FIG. 2

Tumour showing bundles and whorls of spindle cells including eosinophilic elongated strap and tennis racquet shaped cells centrally (haematoxylin and eosin ×80).

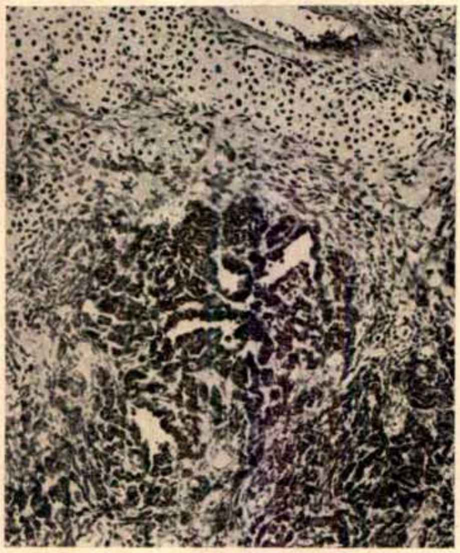

FIG. 3

Tumour showing chondroblastic differentiation with adjacent tubulo-papillary differentiation (haematoxylin and eosin ×13).

and a few intervening atypical spindle cells. The tubal epithelium showed no evidence of neoplastic change. Multiple histological sections failed to reveal evidence of neoplastic change in endometrium or cervix. The left Fallopian tube, left ovary and attached broad ligament showed no evidence of neoplasm.

The patient made a good recovery and was discharged home. One month later she developed ascites. She had an abdominal paracentesis followed by the instillation of 22·5 mg of thiotepa. This caused a lot of discomfort when injected and the dose given was only half that intended. A second abdominal paracentesis was performed two weeks later because of recurrent ascites. Two litres of fluid were removed but no thiotepa was given. The patient died at home 15 weeks after she first presented.

DISCUSSION

Mixed mesenchymal sarcomas (mixed mesodermal tumours) may arise from the endometrium, cervix, vagina (Taylor, 1958), ovary (Fox and Langley, 1975) or, very rarely, the Fallopian tube (Williams and Woodruff, 1963). Origin from the broad ligament itself has been doubted (Meikle, 1936) and, in general, malignant tumours at this site are regarded as having spread from a uterine or ovarian primary (Haines and Taylor, 1975).

The essential criteria for a diagnosis of genital mixed mesenchymal sarcoma of the broad ligament are the demonstration of definitive histological features and the exclusion of a uterine, ovarian or tubal primary neoplasm. The diagnostic features required are a mixture of carcinoma and sarcoma associated with heterologous elements such as cartilage or rhabdomyoblasts. Many areas in such tumours may be undifferentiated or of carcinomatous appearance hence it is often necessary to examine a large amount of tumour with multiple sections to find the essential histological evidence. Our patient showed carcinoma and sarcoma with heterologous elements in the original specimen but not in the tumour attached to the hysterectomy specimen obtained at the second operation.

Anatomically the tumour seemed to arise from the fimbrial end of the right Fallopian tube and the broad ligament itself. Mixed mesenchymal sarcomas of the female genital tract probably arise from Müllerian mesoderm or closely related tissue (Taylor, 1958). Their possible origin from foci of endometriosis is supported by some case reports (Decker *et al*, 1968). Our patient had no evidence of endometriosis and it appears likely that the mixed mesenchymal sarcoma arose from Müllerian remnants in the lateral part of the broad ligament adjacent to the Fallopian tube. The same was the case for the patients described by Wurtz (1949) and Ober and Edgecombe (1954).

The prognosis of mixed mesenchymal sarcoma of the uterus (Chuang *et al*, 1970) and ovary (Fox and Langley, 1975) is very poor. Treatment usually consists of surgery and radiotherapy (Decker *et al*, 1968). The patient described by Williams and Woodruff (1963) survived for 4½ years after total abdominal hysterectomy and bilateral salpingo-oophorectomy for a tubal mixed mesenchymal tumour.

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IDIOPATHIC RETROPERITONEAL FIBROSIS INVOLVING THE VAGINA: CASE REPORT

BY

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Summary

A patient with idiopathic retroperitoneal fibrosis involving the vagina and causing urinary symptoms is described. The condition responded satisfactorily to immunosuppressive drugs and steroid.

IDIOPATHIC retroperitoneal fibrosis is a condition of unknown aetiology in which dense fibrous tissues affect all the retroperitoneal systems. It is three times commoner in men than women, with a maximal incidence in the fourth decade. There were reports of 95 patients with the disease up to 1960, the first being presented by Ormond (1948).

The condition may present with back pain and anuria. The fibrosis may involve any part of the ureters, which are displaced medially and compressed but permit the passage of a retrograde ureteric catheter. The response of retroperitoneal fibrosis to immunosuppressive drugs and corticosteroids can be rapid and dramatic.

CASE REPORT

The patient was born in 1926 and was first seen by a general surgeon in April, 1971 at the age of 45 when she complained of severe pain in the right loin for four weeks; the pain was associated with shivering, frequency of micturition and dysuria but there was no haematuria. The patient had had intermittent pain in the right loin for 15 years.

Her past history included two years of antituberculous drug treatment for tuberculous lymph nodes of the neck discovered during childhood. A benign breast cyst had been removed in 1955. She had a dilatation and curettage in 1967, followed two years later by a subtotal hysterectomy for an adenomyoma. The cervix could not be removed abdominally because of adhesions to the bladder and the rectum but was excised by the vaginal route in 1972.

No abnormalities were found on general examination. However, an intravenous pyelogram showed a right hydronephrosis with obstruction at the pelvi-ureteric junction. Both kidneys were normal. Cystoscopy in May, 1971 showed a normal bladder and a retrograde catheter passed easily up to the right kidney. X-rays showed slight narrowing of the ureter at the pelvi-ureteric junction, but insufficient evidence of obstruction to justify surgical exploration.

The patient continued to have intermittent pain in the right loin. An intravenous pyelogram in January, 1972 showed no evidence of obstruction of the right ureter which filled easily.

In July, 1976 the patient complained of postcoital spotting and also of difficulty in passing urine. At cystoscopy, the anterior vaginal wall and bladder were found to be thickened and hard. The trigonal area appeared whitish. Biopsy of the bladder and the vaginal wall showed chronic inflammatory changes.

The patient continued to have an irregular loss per vaginam for 18 months. In January, 1978 she was admitted after a sudden blood loss at home. At this time she also complained of dribbling of urine and nocturia.

General examination now revealed a well-nourished Caucasian woman whose blood pressure was 150/80 mm Hg. Pelvic examination revealed a thickened hard vaginal wall with a sinus at the vault. Stale and offensive blood was present in the vagina.

The haemoglobin was 13·8 g/dl, the leucocyte count $8 \cdot 2 \times 10^9$ g/l and the erythrocyte sedimentation rate 12 mm/hour. The blood urea and electrolytes were normal. A high vaginal swab grew no pathogens and guinea pig inoculation for acid-fast bacilli was negative.

At cystoscopy in January, 1978, the bladder base and upper three-quarters of the vaginal wall were 3 to 4 cm thick; the fibrotic condition also involved the posterior vaginal wall extending to the side wall of the pelvis and the bladder base. Multiple biopsies were taken from the vaginal wall and vault.

These biopsies showed intense perivascular fibrosis with chronic inflammatory cell infiltration. There was a sharp line of demarcation between the involved and normal tissue (see Figs. 1 and 2). The appearance resembled idiopathic retroperitoneal fibrosis.

The patient was given 50 mg of cyclophosphamide and 10 mg of prednisolone, both twice daily. Six weeks later she had no symptoms, and the vaginal vault was soft. The patient was still well and asymptomatic five months later. An intravenous pyelogram in June, 1978 showed no evidence of ureteric obstruction.

DISCUSSION

The differential diagnoses of idiopathic retroperitoneal fibrosis are low grade fibrosarcoma, pelvic carcinoma and chronic inflammatory conditions. In the late stages of idiopathic retroperitoneal fibrosis with clinical ureteric obstruction and anuria, retroperitoneal connective tissue tumours and the rare peri-ureteric plastica (Vest and Barelare, 1953) may be

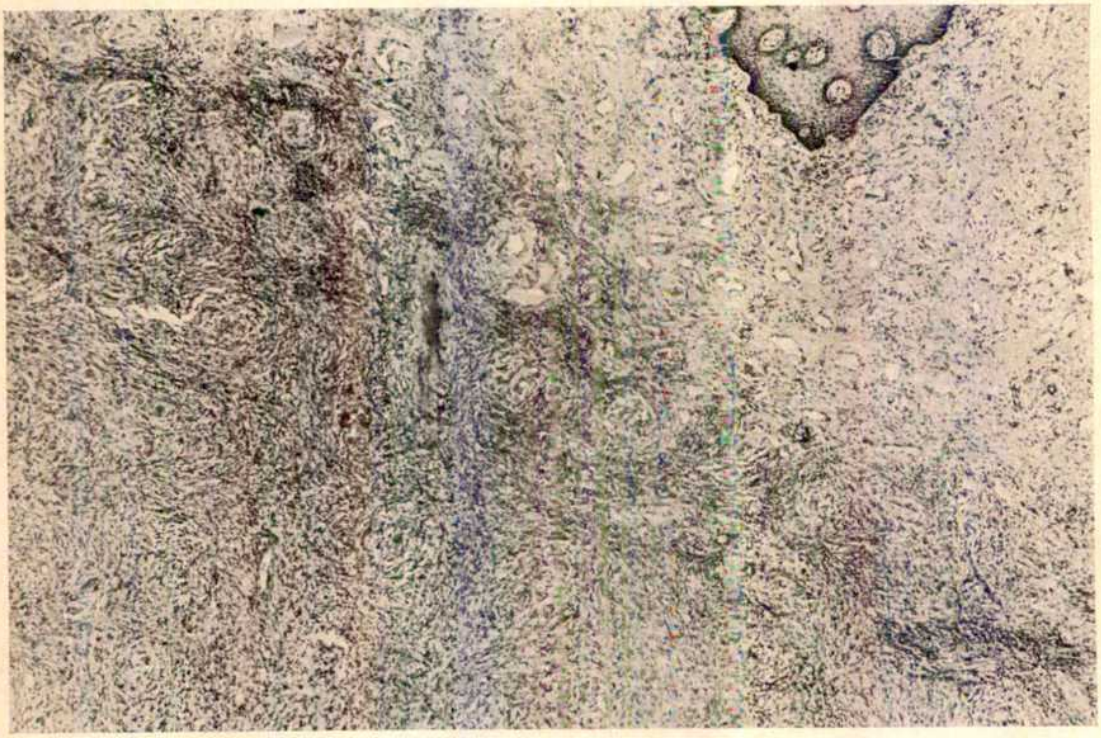

FIG. 1

Retroperitoneal fibrosis. (H. & E. ×40). Vaginal wall biopsy showing a relatively sharp edge to the fibrovascular and chronic inflammatory process (darker zone).

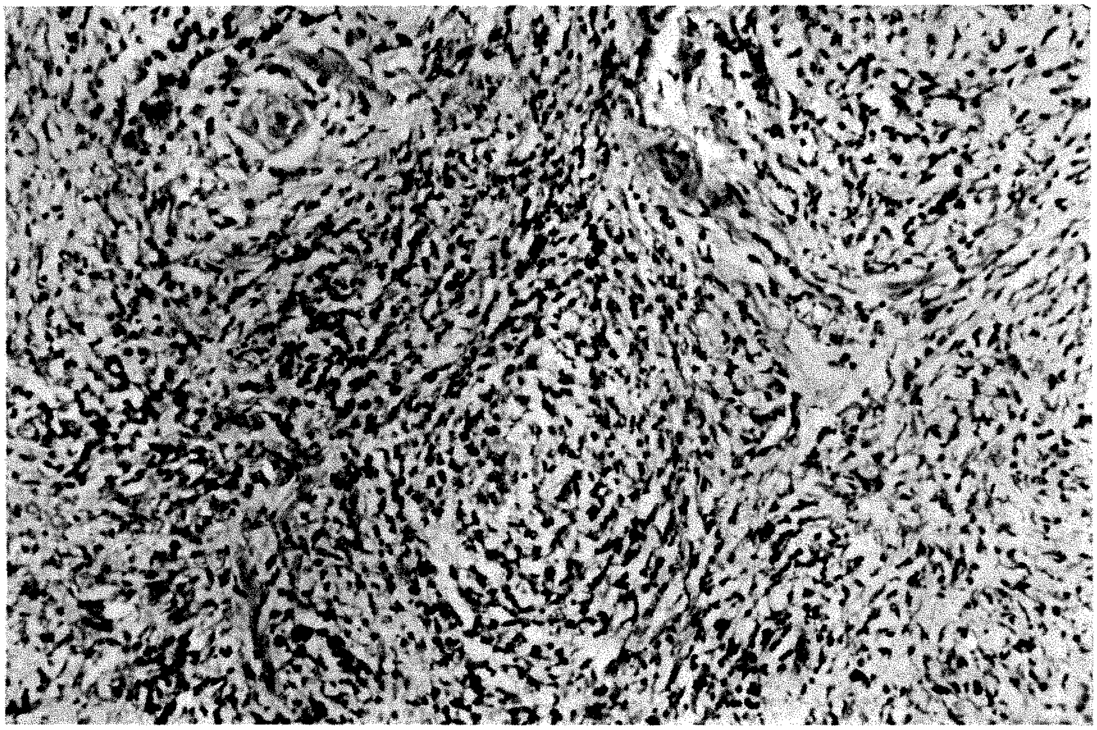

FIG. 2

Retroperitoneal fibrosis. (H. & E. × 160). Vaginal wall biopsy showing the perivascular arrangement of the fibroblastic proliferation and marked chronic inflammatory cell infiltration.

included in the list. To establish a diagnosis multiple biopsies from the affected sites are necessary.

The aetiology of this condition remained obscure. Many authors believe that the disease represents peri-renal fasciitis (Chrisholm *et al*, 1954; Hutch *et al*, 1959). A relationship between retroperitoneal fibrosis and vascular damage, tuberculosis, brucellosis, lymphadenitis and hypersensitivity angiitis have been suggested. Ormond (1948) thought the disease might represent infection of the lymphatics or blood vessels. Houston (1957) suggested that the process was an infection modified by chemotherapy. Graham *et al* (1966) recorded 27 patients who developed retroperitoneal fibrosis whilst taking methysergide. The disease regressed when methysergide was discontinued. De Gennes *et al* (1960) postulated an antigen-antibody reaction with abnormal serum electrophoretic pattern and hyperglobulinaemia. Raper (1956) and Hackett (1958) reported a fatal case with siderosis of the para-aortic nodes and suggested a vascular cause.

The disease involves the urinary and vascular systems causing obstruction by compression (Tinckler and Ross, 1958; Hutch *et al*, 1959). A rectangular area bounded by the renal hila and a line 1 cm lateral to the ureters gets involved. In early stages ureteric involvement may be unilateral but not so later on (Pugh, 1960).

Symptoms include abdominal pain and distension with weight loss, anorexia, vomiting, anuria and low grade pyrexia. Physical examination usually reveals little abnormality. Lower abdominal and flank tenderness may be the only findings. A fixed mass overlying the sacral promontory is occasionally felt.

Microscopically, the appearance consists of plaques composed of dense proliferative fibrous tissue, often infiltrated by chronic inflammatory cells and perivascular arteriolitis. The process may permeate fat and extend to adjacent lymph nodes. The plaques may consist of hyalinized

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PSEUDO-OBSTRUCTION OF THE BOWEL IN PREGNANCY

CASE REPORTS

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Summary

Two patients with intestinal obstruction in pregnancy are presented and discussed. In neither case was there any obvious cause for the intestinal obstruction. Pseudo-obstruction of the bowel has not previously been described as a complication of pregnancy.

THE acute abdomen is always a difficult diagnostic problem in pregnancy and intestinal obstruction is a rare, and frequently undiagnosed cause of pain (Morris, 1965). Various causes of intestinal obstruction complicating pregnancy have been described including adhesions (Svesko and Pisani, 1960) volvulus, intussusception and tumours of the bowel.

Pseudo-obstruction is a diagnosis made by exclusion and has been described as 'where the symptoms and signs of mechanical intestinal obstruction are present, but on further investigation, or at operation, no obstruction is found' (Leading Article, 1973). A variety of associated illnesses have been described: these include renal failure, cardiac failure and myocardial infarction (Leading Article, 1973). We are not aware of any association between pregnancy and pseudo-obstruction, but a similar occurrence has been described after Caesarean Section (Anthony and Wallace, 1962).

CASE REPORTS

Patient 1

A 25-year-old presented to her general practitioner at 34 weeks gestation in her second pregnancy with right sided abdominal pain. Her previous pregnancy had been uneventful and ended at 40 weeks with a normal delivery. There was no past history of abdominal pain, constipation or abdominal surgery.

The initial diagnosis was of urinary tract infection and the patient was treated in the General Practitioner Maternity Unit with ampicillin. A mid-stream specimen of urine was sterile.

After three days the pain became more intense and colicky in nature. The patient vomited and became more distended with absolute constipation. She was transferred to the Consultant Unit and a diagnosis of intestinal obstruction was made on clinical and radiological signs.

The patient was treated with intravenous fluids and nasogastric suction. After 24 hours she had a laparotomy.

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At laparotomy the large bowel was widely dilated as far as 8 cm below the splenic flexure where the calibre was normal again. There were no adhesions, no volvulus, intussusception or tumour. There were no signs of peritonitis. The caecum was grossly distended with some tearing of the serosal peritoneum. The large bowel was decompressed and a caecostomy performed.

After 24 hours spontaneous labour ensued and a male infant weighing 2·350 g was delivered under epidural block with Kielland's forceps. A subsequent barium enema has shown the large bowel to be normal to the site of the caecostomy and the latter was formally closed one month after laparotomy.

The patient has since been asymptomatic but there has been no further pregnancy.

Patient 2

A 24-year-old woman was admitted at 27 weeks gestation in her second pregnancy, for rest because of a twin pregnancy. Her first pregnancy had been terminated for social reasons at ten weeks. There was a past history of meningitis, appendicitis and jaundice.

The maturity of the present pregnancy had been confirmed by ultrasound at thirteen weeks and at which time twins had been diagnosed.

Following admission the patient complained of epigastric discomfort which responded to antacids. A glucose tolerance test, performed because of a family history of diabetes, showed gestational diabetes and the patient was treated with a 150 g carbohydrate diet.

At 29 weeks gestation, uterine contractions were noted and the patient was started on oral salbutamol. At 30 weeks these contractions became more intense and required intravenous salbutamol. The uterine contractions ceased but the patient vomited and developed abdominal distension with increasingly severe abdominal pain. The clinical signs were those of intestinal obstruction, and an abdominal X-ray revealed dilated loops of bowel.

Conservative management with intravenous fluids and nasogastric suction was started. After 24 hours the pain became more severe and uterine contractions recommenced. Laparotomy was performed.

At operation the twins were delivered by routine lower segment Caesarean section and weighed 1·4 kg and 1·3 kg. The transverse and descending colon were grossly dilated down to the level of the pelvic brim. The rectum was empty and collapsed. There were no adhesions, volvulus or other obvious cause for the obstruction. The bowel was deflated with a tube passed per rectum and the wound was closed in the usual position.

Postoperatively, the patient showed clinical signs of obstruction for four days but then made a good recovery. A subsequent sigmoidoscopy has been normal and the patient is now asymptomatic.

COMMENT

The most common cause of intestinal obstruction in pregnancy appears to be adhesions (Svesko and Pisani, 1960; Morris, 1965) and occurs most commonly between the fifth and eighth months of pregnancy (Goldthorpe, 1966). Compression of the large bowel by an incarcerated uterus after delivery has been described (Anthony and Wallace, 1962). It may be that this was a factor in patient 2, with a large uterus compressing the bowel at the pelvic brim. If, however, this was the case it seems strange that it is not a common event. A variety of drugs were also being taken by patient 2. We do not, however, know of any previous cases where salbutamol or antacids have caused obstruction of the bowel. In patient 1, the bowel was distended to a degree where rupture seemed likely, hence the defunctioning caecostomy. This feature has been described by other authors in intestinal obstruction in pregnancy (Anthony and Wallace, 1962; Morris, 1965).

One of the problems of diagnosing intestinal obstruction in pregnancy, is that vomiting and constipation, together with vague abdominal pains are so common. Nevertheless, had these patients not been pregnant, operation might have been performed sooner; in other patients described, excessive delay has proved fatal (Morris, 1965).

At the time of surgery, delivery by Caesarean Section may be required to allow a proper laparotomy. This was the case in patient 2. In any event, premature labour was probably precipitated by surgery in patient 1 and nothing was gained by not delivering the baby abdominally.

ACKNOWLEDGEMENT

We thank Mr M. J. Hare for permission to report patient 2 and for his help in preparing the paper.

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BOOK REVIEWS

Choriocarcinoma and Related Gestational Trophoblastic Tumours in Women. By R. HERTZ. Pp ix+170, illustrated. Raven Press, New York, 1978. Price:

DR HERTZ has written a very readable account about this group of diseases. His approach is summarized in the Preface, when he comments 'this account is not an exhaustive one, for it is meant to teach rather than to tire'. I think this short book serves as a good introduction to these tumours, but does not include some of the more recent information about them or improvements in their treatment. In the first chapter on the trophoblast, he emphasizes some of the parallels in behaviour between the normal and the malignant trophoblast. He correctly emphasizes that the gestational trophoblastic tumours form a spectrum of disease with widely varying biological expression. He also states clearly that the clinical behaviour of a particular tumour need not bear a close relationship to the original histology, since it is the part of the disease that is actually growing in the patient that is more important than the sample that was removed from the uterus. He presents some of the epidemiological data which indicates that there are probably marked differences in incidence in trophoblastic tumours around the world. He devotes a full chapter to hormonal and biochemical indicators of these tumours. The progressive improvement in measurement of human chorionic gonadotrophin (and now the beta assay which is specific for hCG) has made a major impact on following these patients and monitoring their response to treatment. Perhaps the weakest chapter is that on Management and Surveillance. The author points out that some centres use prophylactic chemotherapy in patients with hydatidiform mole, while other centres are more selective in the patients they treat. He does not make it sufficiently clear that in order to make a case for prophylactic chemotherapy, where a significant fraction of the patients would cure themselves of their own disease, one has to show that there are more therapeutic failures in centres using an expectant policy. There is no clear-cut evidence that the results of prophylactic chemotherapy are superior in terms of overall survival. More emphasis should also be placed on the stratification of patients into 'risk groups' so that their chemotherapy can be planned appropriately. In the section on the side-effects of methotrexate, no mention is made of the reversal of its action by folinic acid to prevent toxicity. The clinical pharmacology of methotrexate is sufficiently understood for it to be given safely with minimal side effects. The last chapter is one of the most interesting, which indicates how the various threads of information resulted in the recognition of folic acid in the body, and the subsequent development of antifolates as cytotoxic agents which are so useful in treating gestational trophoblastic tumours and certain other cancers.

E. S. Newlands

Novel Aspects of Reproductive Physiology. Edited by C. H. SPILMAN and J. W. WILKS. Pp 397, illustrated. John Wiley & Sons Ltd., Baffius Lane, Chichester, 1978. Price: £14.00.

ALMOST inevitably, this book is the record of a symposium, in this case a Brook Lodge Workshop supported by the Upjohn Company. As a result the company's employees are generously represented in the list of authors but it does mean that the articles are all invited contributions by writers with claims to authority in their subject. Obstetricians will find the central group of five papers on embryo-uterine interactions the most interesting. These are expositions on topics about which we don't hear much. They are likely to affect our practice in the future and it is a good idea to make a passing acquaintance before the jargon becomes too specialized to understand. Embryo-uterine interactions in this context means the biochemical signals which pass between the endometrium and the conceptus. For the most part they are transmitted by proteins and it was surprising to find how far biochemists have gone in identifying, isolating, characterizing and measuring these substances. Attention appears to have been first directed to proteins produced by the mother. There are good reviews by Beier and by Feigelson on uteroglobulin, a protein principally secreted by the endometrium but also produced by the oviduct and the epithelium of the lung and gut. It was isolated from rabbit uteri and might be supposed to be peculiar to that species. Feigelson has now unequivocally identified a similar protein from human uterine fluid and it is likely that interest in this protein will now increase briskly. Its secretion in the uterus is controlled by progesterone and it binds this steroid, albeit under unphysiological circumstances which make it unlikely that uteroglobulin functions as a progesterone transport protein. Hans Denker argues a good case for the role of trophoblastic factors, principally enzymes in the covering of the blastocyst, as opposed to uterine factors in implantation. His thesis is that uterine factors are mainly inhibitors of implantation and that implantation occurs when they decrease or are overcome by the trophoblastic factors. The importance of this concept to research on contraception is obvious. The principal trophoblastic factor identified by Denker is a trypsin-like proteinase. Terry Baker writes an epilogue which is a *cri de coeur* about the multiplicity of proteins now found to be playing a part in hormone action. Perhaps he is right; we are now in the stage with proteins where we were with steroids a decade or two ago when new steroid hormones were being isolated almost daily. In the end many of these proved to be metabolites or involved in physiologically insignificant alterative pathways. But which protein is which?

A. Klopper

Diagnosis and Treatment of Functional Infertility. By B. LUNENFELD and V. INSLER. Pp viii+202, with 54 illustrations, 32 in colour and 32 tables. Grosse Verlag, Berlin, 1978.

THIS is a handy and easily read paperback written by two of the most experienced practitioners and researchers in the field of infertility, with the collaboration of Dr M. Glezerman in the section on male infertility. It concerns the medical 'functional' problems of ovulatory failure, impaired sperm progression in cervical mucus, and defective semen. It does not deal with the surgical problems of the Fallopian tubes or male genital ducts. The authors aim their book at both students of reproductive physiology and medical practitioners. For the latter there could sometimes be clearer practical interpretation, especially concerning some aspects of male infertility, of otherwise excellently reviewed information. I am surprised at the space given to discarded treatment with oestrogens and progestogens. Also, the discussion on prolactin has, inevitably, been overtaken by the rapid recent development of clinical information about it, and the polycystic ovary syndrome has not received any particular attention. That, however, is as far as it is possible to criticize this otherwise excellent little book. There are easily understood sections on the normal control of the ovaries, cervix and testicles, and also of erection and ejaculation. There are most useful reviews of the various drugs used to induce ovulation, with a particularly valuable section on, as might be expected from these authors, gonadotrophin therapy. There is an excellent section on cervical mucus. Also, a beautifully logical diagnostic scheme for male infertility, and although the therapeutic tools remain largely wanting, at least the approaches to the problem have been unfogged for the average reader. The book is liberally illustrated with clinical examples and step-by-step diagnostic schemes made simpler by the use of colour, which every teacher will want to copy. Finally, there is a profuse bibliography to be explored. Indeed as a reference book it is remarkable, being also easy to read and within the compass of the busiest gynaecologist.

M. G. R. Hull

Practical Abdominal Ultrasound. Edited by C. METREWELI. Pp v+125, illustrated. Butler & Tanner Ltd, London, 1978. Price: £12.50.

IN this preface the author writes that this is a 'book written by a beginner for beginners', and in the forward Ian Donald suggests that it is 'a sort of users manual giving clear advice on what makes a good picture or a bad one and how to make sense out of the results'. These two statements indicate the content of the book and also its use. However, the author denigrates himself in suggesting that he is a beginner in the use of ultrasound since his experience in the practical aspects of carrying out scans and assessing the results is obviously immense, and he imparts to the reader much of his expertise. It is important to the beginner that he is not only au fait with the jargon of ultrasound, but also has an awareness of various scanning techniques and the different types of apparatus used. These are comprehensively covered in the first few chapters of the book. Useful hints on how to obtain the best scans are given. Illustrations of pitfalls the operator may encounter either due to faulty use of the machine or wrong interpretation of pictures due to artefacts are particularly helpful. As suggested by its title, the book concentrates on ultrasound examination of the abdomen and includes detailed chapters on the kidney, the gall bladder, the liver and the pancreas. Gynaecological masses are illustrated, and two chapters are devoted to the examination of the abdomen in pregnancy. Useful graphs of fetal indices related to gestational age are included. I remain unconvinced that a placenta may at one stage in pregnancy cover the cervical os and later migrate to the upper segment of the uterus as is diagramatically shown. The real value of the book is in its many illustrations. Particularly helpful are the reproductions of ultrasonic scans with diagramatic explanations alongside, although occasionally the 'eye of faith' may be necessary to discern some of the detail. The 114 plates at the end of the book might have proven of greater value had they been included within the chapters to which they referred and with accompanying diagrams. The drawings used to clarify the text may depress the beginner since similar scans cannot be reproduced without considerable experience and skill. The book is beautifully produced and makes a splendid addition to the increasing number of text books devoted to diagnostic ultrasound. It can be recommended to all clinicians interested in this diagnostic science whether beginners or experienced operators.

J. MacVicar

Gynecologic Oncology Case Studies. By S. LIFSHITZ and H. J. BUCHSBAUM. Pp 215, with 38 figs and 40 tables. Henry Kimpton Publishers, London, 1978. Price: £10.50.

THIS is an interesting method of postgraduate instruction which has much to commend it for a sub-speciality. It would be useful reading for the postgraduate student and for those called upon to manage cases of malignant disease but who do not have access to advice from oncological specialists. It is to be hoped that the latter are a diminishing number. The format is strictly clinical but makes use of abbreviations without a glossary which might be unhelpful to a non-American reader. Again the 'mini' multiple choice element introduced into a factual description could be less relevant to those unfamiliar with this approach. The references tend to be repetitive and might be better grouped at the end of each section. Despite these criticisms the presentation can only be instructive and enlightening and will give the postgraduate student a logical and sensible approach to gynaecological oncology on which to base further studies and with which to manage clinical problems.

R. M. Feroze

Cervical Pathology and Colposcopy. Edited by E. BURGHARDT, E. HOLZER and J. A. JORDAN. Pp xiv+146, with 65 figs and 41 tables. Georg Thieme Publishers, Stuttgart, 1978. Price: DM 48.

THE Second World Congress for Cervical Pathology and Colposcopy was held in Graz, Austria, in October 1975, a year which also marked the 50th anniversary of colposcopy. There were 130 lectures as well as round-table discussions and study groups. This book contains the main lectures on the main topics and the summaries from the round-table discussions and study groups. As always seems to be the way with such publications, the book has appeared nearly three years after the Congress and only a few months before the Third World Congress for Cervical Pathology and Colposcopy, so that much of the material has been superseded by more up-to-date work. There are sections covering most aspects of cervical pathology and colposcopy, including the stilboestrol problem, immunology, epidemiology, microcarcinoma, the transformation zone and dysplasia. However, there is little information which is not available in journals or other monographs. The opening chapter, on the development of colposcopy over the past 50 years and the present situation in various countries is perhaps the exception, being of considerable background interest to colposcopists. To those who went to the Congress, this book serves as a reminder of what was presented but on the whole it cannot be recommended as a useful source of information.

M. C. Anderson

Clinics in Obstetrics and Gynaecology. Hypertensive States in Pregnancy. Vol. 4, No. 3. Edited by E. M. SYMONDS. Pp vii+762, with 69 figs and 16 tables. W. B. Saunders Co. Ltd, Ist Anne's Road, Eastbourne, East Sussex BN21 3UN, 1977. Price: £7.50.

'CLARIFICATION of the present inconsistencies requires further research'. Not the phrase for a textbook, but appropriate for a volume such as this which reviews concepts while they are evolving, as well as those which are firmly established. Clotting, renin-angiotensin and prostaglandins are growth areas which are fully covered: renal changes and the different types of proteinuria are established, requiring only brief mention. This attitude pervades the whole book and makes it one to be read in its entirety. Because much of the material is relatively new, some chapters may need to be read more than once: this is repaid by increased understanding both of that topic, and also of related ones. This volume is a readable review of current thought in pregnancy hypertension. Wisely there is no chapter specifically on aetiology, but the rest is there and worth reading.

I. R. McFadyen

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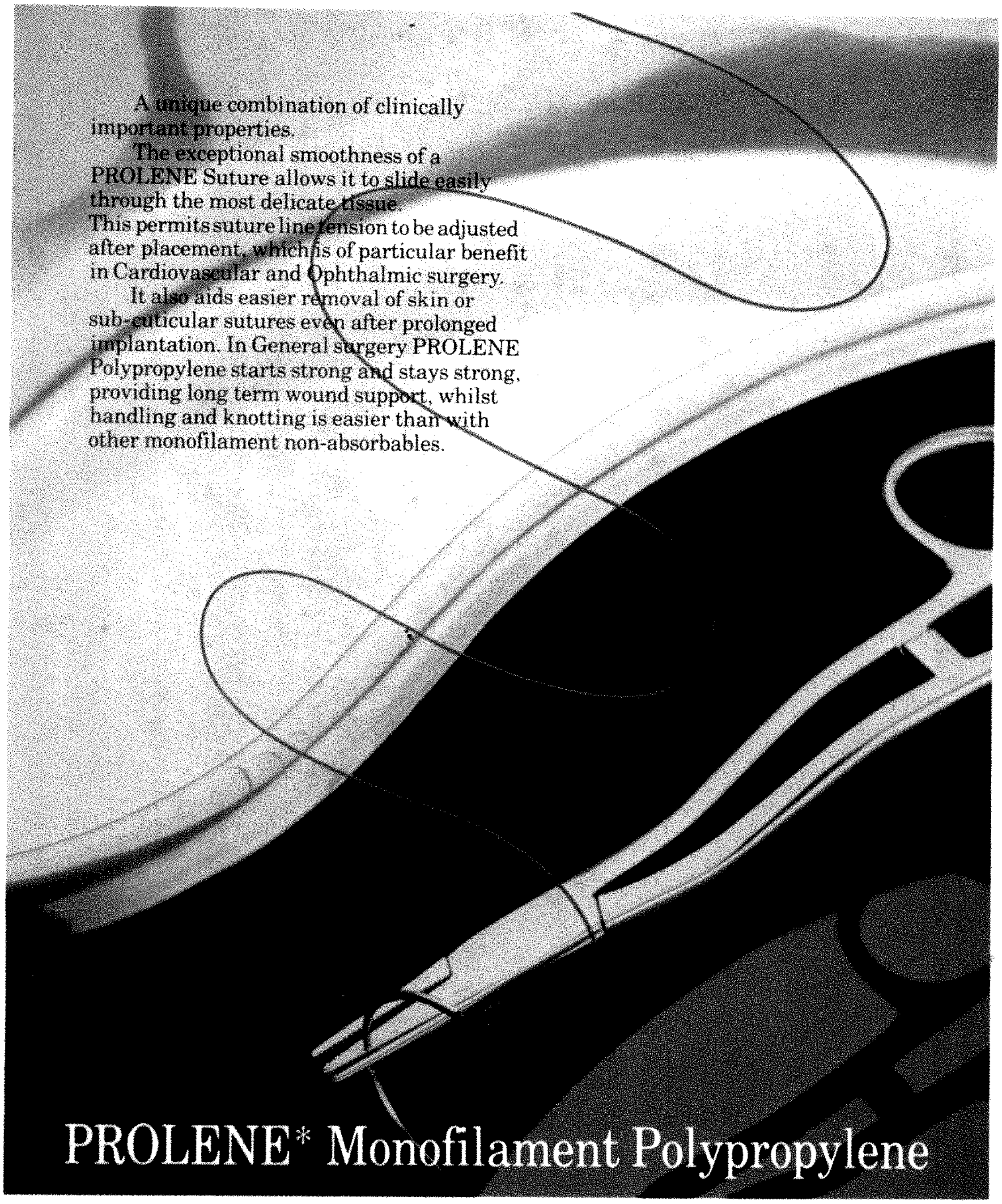
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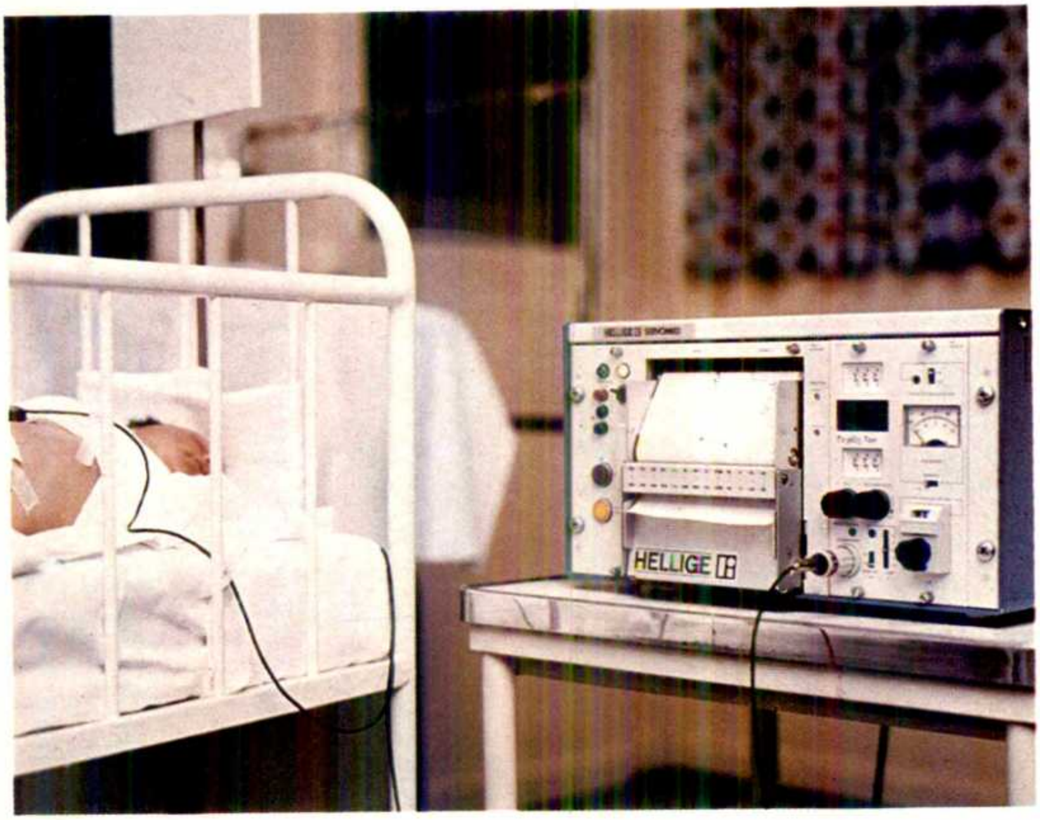

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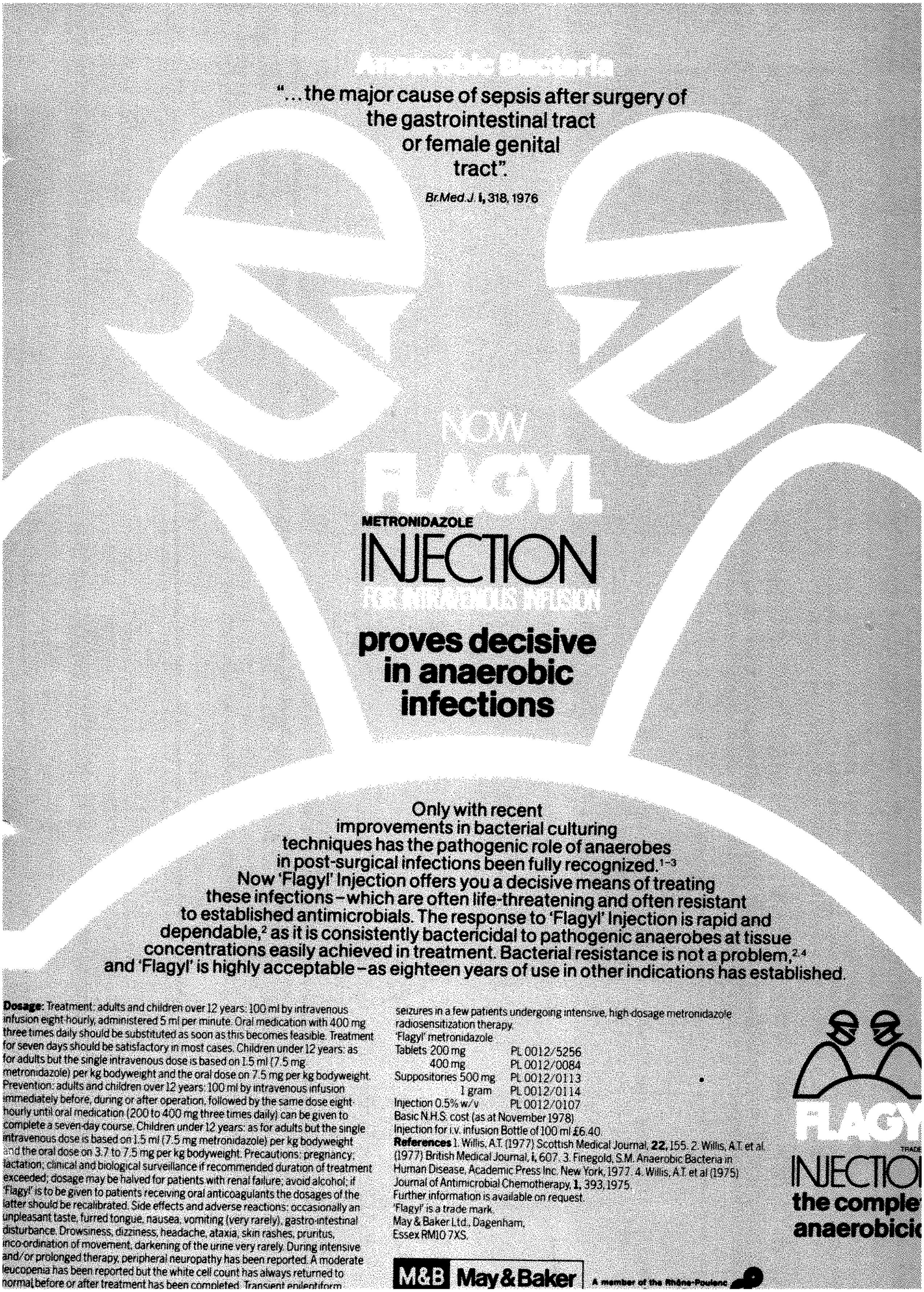

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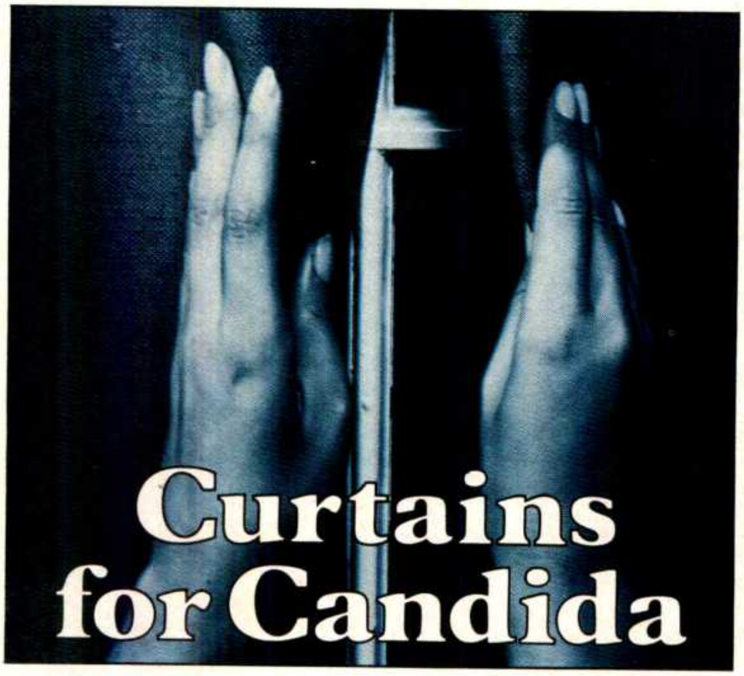


In just 3 nights

Ecostatin™

Econazole nitrate

Ecostatin™ eliminates candidal vaginitis in just 3 nights

- ✱ it is the most active fungicide against a wide range of pathogens[1]
- ✱ it combines potent fungistatic and fungicidal properties[2]

Ecostatin also ensures

- ✱ fast relief of symptoms
- ✱ no resistance[3]
- ✱ patient compliance

Ecostatin is also available as a cream for a wide range of dermatomycoses.

Prescribing Information

PRESENTATION

White, opaque oval pessaries each containing 150mg econazole nitrate in an hydrogenated vegetable oil base.

USES

Actions Econazole nitrate has a broad spectrum of antifungal activity. It is highly active against Candida albicans and other Candida species and is effective in controlling infections of the vagina and vulva caused by such organisms (thrush).

Indications Vulvovaginal candidosis.

DOSAGE AND ADMINISTRATION

One pessary to be inserted at bedtime for 3 consecutive nights. Administration should be continued even if menstruation occurs, and despite the disappearance of signs and symptoms of the infection. The pessary should be inserted high into the vagina while the patient is supine. Although a three day course of therapy usually suffices it may be necessary to institute a second course of therapy.

CONTRA-INDICATIONS, WARNINGS, etc.

Contra-indications None known.

Precautions Ecostatin pessaries are effective in the candidal vaginitis associated with pregnancy. However, as with other agents, it should not be used during the first trimester of pregnancy unless the physician deems it's use essential for the welfare of the patient.

Side Effects Very occasionally patients may complain of discomfort; this is usually transitory and disappears with continued treatment. Rarely is it necessary to discontinue econazole pessary treatment.

PHARMACEUTICAL PRECAUTIONS

Storage Store at room temperature.

LEGAL CATEGORY POM

PACKAGE QUANTITIES Packs of three pessaries with applicator.

Price – 3 pessaries with applicator £3.60

Product Licence Number 0085/0013.

References

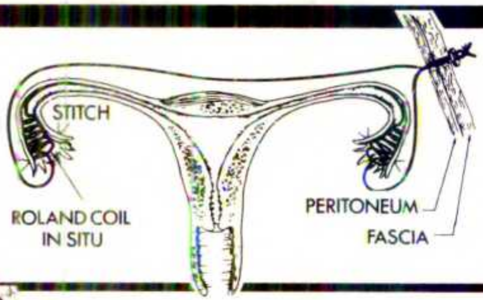
1. Avram, A. et Grupper, Ch. Bulletin de la Société Française de Mycologie Médicale 1975 Tome IV (2).
2. Preusser, H.J. et al. Research Report 1974. On file Cilag-Chemie.
3. Schweisfurth, R. Research Report 1974. On file Cilag-Chemie.

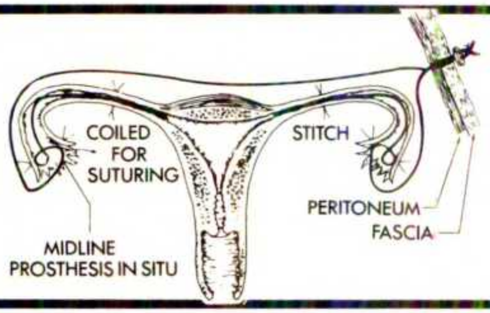
Ecostatin is a registered trade mark for econazole nitrate and FAIR Laboratories is the registered user.

Further information is available from:
FAIR Laboratories,
179 Heath Road, Twickenham TW1 4BJ

kli tuboplasty surgical prosthesis

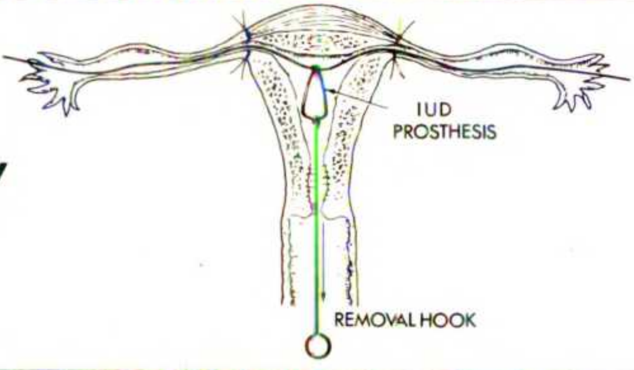
Stent for Fimbrial Tuboplasty





Stent for Midline Tuboplasty

Stent for Cornual Tuboplasty



Three disposable prostheses designed to maintain tubal patency following fimbrial, midline or cornual repair.

X-ray opaque. Simple technique for removal.

Write for more details of the prostheses, and information on our tubal surgical instruments.

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4 Dollis Park, London N3 1HG.
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BY APPOINTMENT
TO H.M. THE QUEEN
SUPPLIERS OF SURGICAL EQUIPMENT
CORY BROS (HOSPITAL CONTRACTS) CO. LTD
LONDON

Prescribing Information

Presentation
White egg-shaped pessaries each containing 150mg econazole nitrate and melting at 37°C.
Uses
Vaginitis due to Candida albicans and other yeasts.
Dosage and Administration
One pessary should be inserted high into the vagina each evening for three consecutive days.
Contra-indications, Warnings etc.
Contra-indications:
None known.
Side-effects:
None known.
Overdosage:
Not applicable.
Precautions:
Hypersensitivity has never been recorded, but if it should occur, administration of the product should be discontinued.
Gyno-Pevaryl* 150 vaginal pessaries are effective in the treatment of vaginal candidiasis associated with pregnancy but as with any new product, caution should be exercised when prescribing during the first trimester.
Pharmaceutical Precautions
Store in a cool place.
Legal Category
Prescription only (POM).
Package Quantities
Each pack contains 3 pessaries and a patient instruction leaflet.
The pessaries are individually sealed in a white plastic strip marked **Gyno-Pevaryl*** 150 at regular intervals.
Further Information
Anogenital hygiene is important to help prevent re-infection.
PL0076/0058
Basic NHS Price – £3.60
Name and address of Licensee
Ortho Pharmaceutical Limited, P.O. Box 79, Saunderton, High Wycombe, Buckinghamshire HP14 4HJ, England.
Further information and data sheet available on request.

Ortho

NEW PRODUCT

NEW PRODUCT

The quick way to say Gyno-Pevaryl*

Vaginal Pessaries

GP

The effective 3 day, 3 pessary treatment for vaginal candidiasis

Rapid symptomatic relief Burning pruritus and discharge promptly subside on therapy.
Effective in over 85% of cases – in only 3 days[1] Three days are all it takes to clear the condition in over 85% of cases.[1]
Better patient compliance Three rapidly dissolving soft-wax pessaries inserted one at night for three days, effectively minimise the possibility of recurrence through non-compliance by reducing the therapy to its simplest form ever. **Gyno-Pevaryl* 150** vaginal pessaries – easy, quick and effective in the treatment of vaginal candidiasis.

NEW
Gyno-Pevaryl* 150

Vaginal Pessaries **Econazole Nitrate 150mg.**

new-generation antifungal

the quick way to treat vaginal candidiasis

*Trademark ©OPL 1978 Reference: 1. Balmer, J.A. (1976). Am J. Obstet.Gynae. 126, 4, 436-441.

more convenient labour induction

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for the patient...for the staff

Prostin E2
(dinoprostone)

For effective induction of labour without the inconvenience of intravenous administration.

Prostin E2 Tablets are available only to hospitals and clinics with specialised obstetric units and should only be used where 24-hour resident medical cover is provided.

Full product information available on request.

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UPJOHN LIMITED · CRAWLEY · WEST SUSSEX

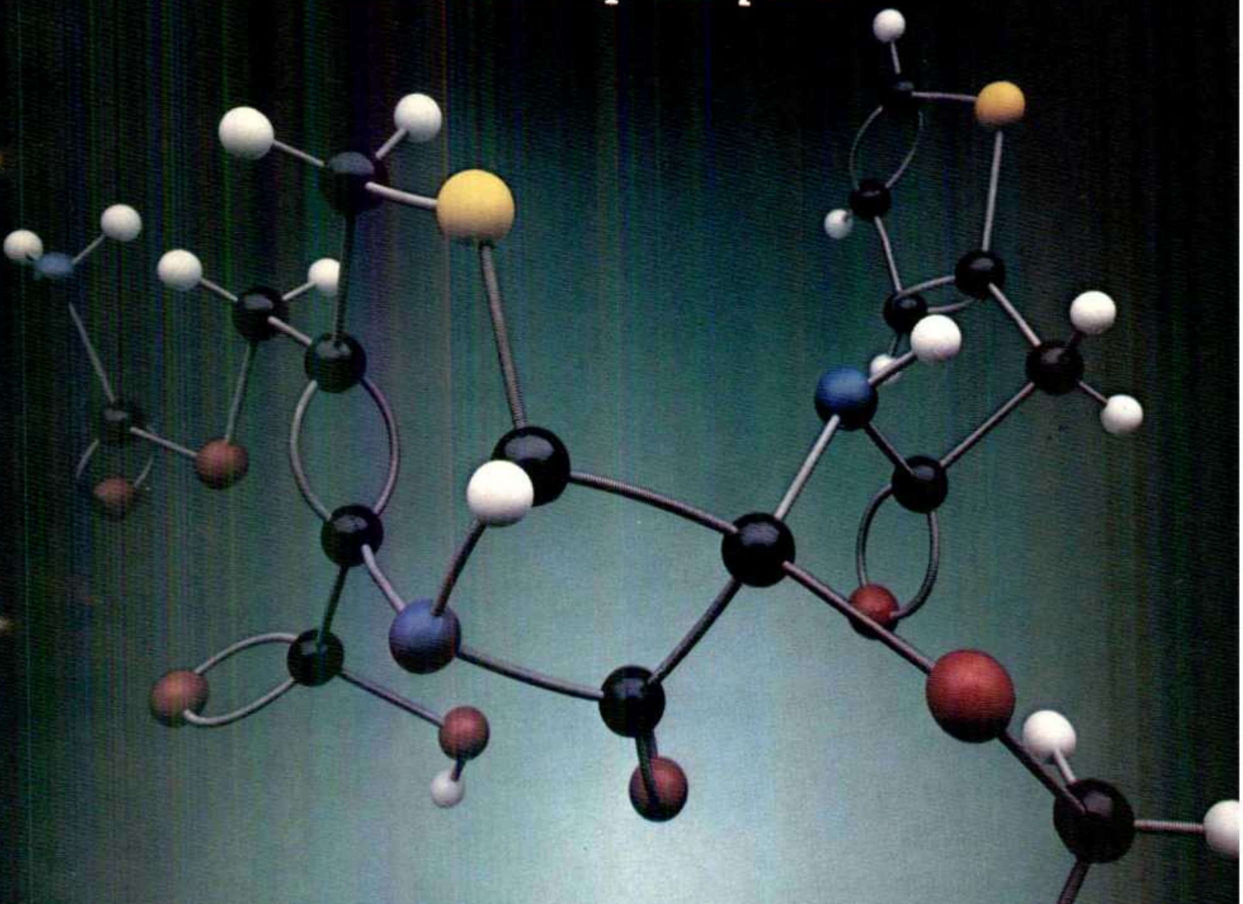
MSD announces the discovery of

Mefoxin®

Cefoxitin sodium, MSD

INJECTION

First of the cephamycins

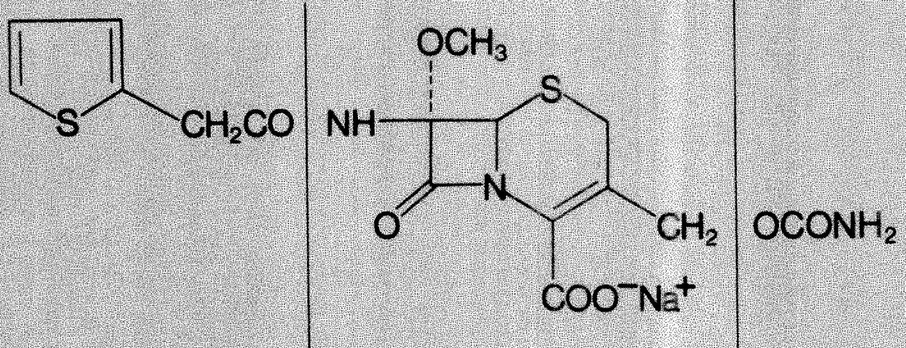


A new class of antibiotics

'Mefoxin' is derived from cephamycin C, a naturally occurring antibiotic substance produced by *Streptomyces lactamdurans*. This naturally occurring antibiotic has been modified in the MSD Research Laboratories to enhance its therapeutic usefulness.

Prescribing information overleaf

‘Mefoxin’ has a unique molecular structure offering outstanding clinical benefits and a specific clinical profile.



Thienylacetyl side-chain:
enhances activity against Gram-positive pathogens

Cephamycin nucleus:
provides bactericidal activity against a wide range of pathogens, Gram-negative in particular. 7α-methoxy group protects against β-lactamase degradation

Carbamoyloxy side-chain:
protects antibiotic from being metabolised

▼Prescribing information

Indications

'Mefoxin' is a broad-spectrum bactericidal antibiotic indicated for the treatment of infections caused by susceptible strains of Gram-positive and Gram-negative pathogens both aerobic and anaerobic

'Mefoxin' has been clinically effective not only in infections due to antibiotic-sensitive organisms, but also in infections due to organisms resistant to one or more of the following antibacterial agents: penicillin, ampicillin, carbenicillin, tetracyclines, erythromycin, chloramphenicol, cephalosporins, kanamycin, gentamicin, tobramycin, and sulphamethoxazole-trimethoprim

Many Gram-negative pathogens are resistant to penicillins and cephalosporins through the action of the β-lactamases which are produced by these pathogens. 'Mefoxin' is remarkably stable in the presence of these bacterial β-lactamases, both penicillinases and cephalosporinases. Hence the clinical efficacy of 'Mefoxin' extends to many infections caused by such pathogens

'Mefoxin' is indicated for the treatment of the following infections caused by sensitive bacteria:
Peritonitis and other intra-abdominal and intrapelvic infections, female genital tract infections, septicaemia, urinary tract infections, respiratory tract infections, bone and joint infections, and skin and soft-tissue infections

'Mefoxin' is indicated for adjunctive therapy in the surgical treatment of infections, including abscesses, infection complicating hollow visceral perforations, cutaneous infections, and infections of serous surfaces whether caused by aerobes, mixed aerobes and anaerobes, or anaerobes

Microbiology

'Mefoxin' is active *in vitro* against

Aerobic bacteria

Gram-positive cocci including *Staphylococci* (including coagulase-positive, coagulase-negative, and penicillinase-producing strains), Group A beta-haemolytic streptococci *(Streptococcus pyogenes)*, Group B beta-haemolytic streptococci *(Streptococcus agalactiae)*, *Streptococcus pneumoniae (Diplococcus pneumoniae)*, other streptococci (except group D streptococci including *faecalis)* *Gram-negative cocci including* *Neisseria gonorrhoeae* (including penicillinase-producing strains), *Neisseria meningitidis* *Gram-negative rods (facultative anaerobes) including* *Escherichia coli*, *Klebsiella pneumoniae*, Klebsiella spp., *Proteus mirabilis*, *Proteus* (indole positive) *Proteus vulgaris*, *Proteus rettgeri*, *Proteus morganii*, *Haemophilus influenzae*, *Serratia marcescens*, Providencia spp., Salmonella and Shigella spp.

Anaerobic bacteria

Gram-positive cocci including Peptococcus spp., Peptostreptococcus spp., Micro-aerophilic streptococcus *Gram-positive rods including* *Clostridium perfringens*, Clostridium spp., Eubacterium spp., *Propionibacterium acnes* *Gram-negative cocci including* Veillonella spp. *Gram-negative rods including* *Bacteroides fragilis*, *Bacteroides melaninogenicus*, Bacteroides spp. (including both penicillin-susceptible and penicillin-resistant strains), Fusobacterium spp.

'Mefoxin' is active against some strains of the following bacteria: *Acinetobacter calcoaceticus var. anitratum (Herellea vaginicola)*, *Acinetobacter calcoaceticus var. lwoffi (Mima polymorpha)*, *Alcaligenes faecalis*, Citrobacter spp. and Flavobacterium spp., Enterobacter spp.

'Mefoxin' is not active against Pseudomonas, most strains of enterococci and many strains of *Enterobacter cloacae*

Dosage and administration

'Mefoxin' may be administered intravenously or intramuscularly. (See reconstitution directions for each route below.) Dosage and route of administration should be determined by severity of infection, susceptibility of the causative organisms, and condition of the patient

Therapy may be started while awaiting the results of susceptibility testing

Dosage: The usual adult dosage is 1 g or 2 g of 'Mefoxin' every eight hours. (See chart.)

In adults with renal insufficiency an initial loading dose of 1 g or 2 g may be given. After a loading dose the following recommendations

Usual adult dosage

Type of infection	Dose	Frequency	Total daily dosage
Uncomplicated	1 g	Every 8 hours (occasionally every 6 hours)	3 g (4 g)
Moderately severe or severe	2 g	Every 8 hours (occasionally every 6 hours)	6 g (8 g)
Infections generally needing antibiotics in higher dosage	3 g (2 g)	Every 6 hours (Every 4 hours)	12 g

Maintenance dosage of 'Mefoxin' in adults with reduced renal function

Renal function	Creatinine clearance (ml/min)	Dose	Frequency
Mild impairment	50-30	1-2 g	Every 8-12 hours
Moderate impairment	29-10	1-2 g	Every 12-24 hour
Severe impairment	9-5	0·5-1 g	Every 12-24 hour
Essentially no function	< 5	0·5-1 g	Every 24-48 hour

In the patients undergoing haemodialysis, the loading dose of 1 to 2 g should be given after each haemodialysis, and the maintenanc dose should be given as indicated in the table above

Intravenous administration: reconstitute 'Mefoxin' with Water for Injection BP. 1 g is soluble in 2 ml. Although 'Mefoxin' is very soluble, for intravenous use it is preferable to add 10 ml of Water f Injection BP to the 1 g vial or the 2 g vial. Shake to dissolve and th withdraw entire contents of vial into syringe

Outstanding clinical benefits

Wide-ranging activity against Gram-negative and Gram-positive pathogens, anaerobes as well as aerobes.

Unusually high resistance to β-lactamase degradation.

Highly active against *Bacteroides fragilis* and strains of *E.coli*, Serratia, Klebsiella spp. and Proteus spp. (including indole positive strains) resistant to other antibacterial agents.

Offers high levels of active antibiotic in the body.

A record of relative safety comparable to the penicillins.

Prime indications include:

– Peritonitis, and other intra-abdominal and intrapelvic infections.
– Female genital tract infections
– Septicaemia
– Skin and soft tissue infections
– Bone and joint infections
– Urinary tract infections
– Respiratory tract infections (e.g. inhalation pneumonia)

Specific clinical profile

'Mefoxin' is exceptionally valuable wherever Gram-negative pathogens and anaerobes are involved in mixed infections. When mixed infections are suspected, therapy may be started while awaiting the results of susceptibility testing.

Mefoxin®

Cefoxitin sodium, MSD

INJECTION

First of the cephamycins

►

the vein over a period of 3 to 5 minutes or may be given through the tubing when the patient is receiving parenteral solutions

An intermittent intravenous infusion of 'Mefoxin' may be employed when large amounts of fluid are to be given. However, during infusion of the solution containing 'Mefoxin' it may be advisable temporarily to discontinue administration of any other infusion solution at the same site (by using an appropriate IV infusion set)

A solution of 'Mefoxin' may also be given by continuous intravenous infusion (see below for compatibility and stability)

Intramuscular administration ONLY

Reconstitute 'Mefoxin' 1 g with 2 ml of Water for Injection BP or 0.5% or 1% lignocaine hydrochloride (without adrenaline) solution. 'Mefoxin' is given by deep injection into a large muscle mass. Avoid injection into a blood vessel

Compatibility and stability

A solution of 'Mefoxin' in Water for Injection BP may be added to the following solutions: 0.9% Sodium Chloride Injection BP, 5% or 10% Dextrose Injection BP, Dextrose and Sodium Chloride Injection (5%/0.9%, 5%/0.45%, or 4.3%/0.18%), Lactated Ringer's Injection USP, 5% Dextrose in Lactated Ringer's Injection, 5% or 10% invert sugar in water, 10% invert sugar in saline solution, 4.2% Sodium Bicarbonate Injection BP, M/6 Sodium Lactate Injection BP

'Mefoxin', as constituted to 1 g/10 ml with Water for Injection BP, Water for Injection BP preserved with parabens or benzyl alcohol, 0.9% Sodium Chloride Injection BP or 5% Dextrose Injection BP, maintains satisfactory potency for 24 hours at room temperature, for 96 hours under refrigeration, and for 13 weeks in the frozen state

'Mefoxin' can be administered concomitantly with such antibiotics as gentamicin, tobramycin, carbenicillin or amikacin. When 'Mefoxin' is administered with another antibiotic, they should not be mixed in the same syringe

Contra-indications

'Mefoxin' is contra-indicated in persons who have shown hypersensitivity to cefoxitin. In the absence of clinical experience 'Mefoxin' should not be administered to patients who have shown

Precautions

There is some clinical and laboratory evidence of partial cross-allergenicity between cephamycins and other β-lactam antibiotics, penicillins, and cephalosporins. Severe reactions (including anaphylaxis) have been reported with most β-lactam antibiotics

Before therapy with 'Mefoxin' careful inquiry should be made concerning previous hypersensitivity reactions to β-lactam antibiotics. 'Mefoxin' should be given cautiously to penicillin-allergic patients

Any patient who has demonstrated some form of allergy, particularly to drugs, should receive antibiotics cautiously. If an allergic reaction to 'Mefoxin' occurs, the drug should be discontinued

The total daily dosage should be reduced when 'Mefoxin' is administered to patients with transient or persistent reduction of urinary output due to renal insufficiency (see 'Dosage and administration') because high and prolonged serum antibiotic concentrations can occur from usual doses

Interference with laboratory tests

A false-positive reaction to glucose in the urine may occur with reducing substances but not with the use of specific glucose oxidase methods

Use in pregnancy

Use of the drug in women of childbearing potential requires that the anticipated benefits be weighed against possible hazards. Reproductive and teratogenic studies have been performed in mice and rats and have revealed no evidence of impaired fertility or harm to the foetus due to 'Mefoxin'. There are no controlled studies with 'Mefoxin' in pregnant women

Nursing mothers

'Mefoxin' is excreted in human milk

Infants and children

Pending the outcome of clinical trials in progress, the conditions for use of 'Mefoxin' in infants and children remain to be defined

Side effects

stopping treatment and usually have been mild and transient

Local reactions

Thrombophlebitis has occurred with intravenous administration. Pain, induration and tenderness after intramuscular injections have been reported

Allergic

Maculopapular rash, urticaria, pruritus, eosinophilia, fever and other allergic reactions have been noted rarely

Gastro-intestinal

Diarrhoea has been reported rarely

Blood

Transient leucopenia and neutropenia have been reported. Some individuals, particularly those with azotaemia, may develop positive direct Coombs tests during therapy with 'Mefoxin'

Liver function

Transient elevations in SGOT, SGPT, serum LDH and serum alkaline phosphatase have been reported rarely

Kidney

Elevations in serum creatinine and/or blood urea levels have been observed. The role of 'Mefoxin' in changes in renal function test is difficult to assess, since factors predisposing to pre-renal azotaemia or to impaired renal function usually have been present

Presentation

In vials containing 1 g or 2 g of cefoxitin as the sodium salt. Each gram of cefoxitin contains approximately 51.5 mg (2.2 mEq) sodium

Product licence numbers: 1 g, 0025/0130; 2 g, 0025/0131

Product authorisation numbers: 1 g, PA 35/42/1; 2 g, PA 35/42/2

Issued October 1978

Additional information is available to the medical profession on request

Basic NHS cost is £4.47 for the 1 g vial and £8.94 for the 2 g vial

MSD

MERCK **Merck Sharp & Dohme Limited**

Parlodel in the infertility clinic

bromocriptine

luteal insufficiency
hyperprolactinaemic amenorrhoea
post-pill amenorrhoea
normoprolactinaemic amenorrhoea
ovulatory infertility

At the centre of endocrine research

Parlodel is established as an effective treatment for hyperprolactinaemic amenorrhoea and the re-establishment of gonadal function. Recent evidence supports its use in patients with differing disorders of reproduction who fail to conceive.[1,2,3,4]

Parlodel should be considered as a treatment for infertility when infection or anatomical causes have been excluded

References 1. *British Journal of Obstetrics & Gynaecology*, **84**, 241–253, 1977. 2. *British Medical Journal*, **2**, 694–697, 1975. 3. *British Medical Journal*, **2**, 1179–1181, 1977. 4. *Current Medical Research & Opinion*, **5**, 734–738, 1978. **Indications** Hyperprolactinaemic infertility. Some infertile women without demonstrable hyperprolactinaemia also respond. **Dosage and Administration** Initially 1.25 to 2.5mg nocte; increasing to 2.5mg bd followed by increments of 2.5mg as necessary at 2–3 day intervals. Take with food. **Precautions** Investigate for pituitary tumour. Institute treatment gradually. Withdraw medication immediately pregnancy is suspected. Regular gynaecological assessment is recommended during long term treatment. **Side-Effects** Nausea, postural hypotension, dizziness, headache, vomiting, constipation. **Presentation** 2.5mg tablets of bromocriptine base. **Product Licence Number** 2.5mg tablets PL/0101/0061. **Basic NHS Cost** 30 x 2.5mg tablets £5.93.

Are they Deficient?

50% of women who have symptoms of pessimism, anxiety, dissatisfaction, lethargy and loss of libido associated with the taking of the oral contraceptive pill have been reported to have an absolute deficiency of vitamin B_6 (pyridoxine hydrochloride).*

COMPLOMENT Continus tablets will effectively ensure adequate supplementation of the pyridoxine from a single tablet, taken daily.

Comploment™
Continus tablets

(Controlled release pyridoxine hydrochloride)

Dosage and Administration
One tablet to be taken daily, or as directed by the Physician.

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3.5 pence

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Continus tablets controlled release technology

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*Ref. Lancet (1973), 1, 897

Fetal Pulse Detectors

Hand held model

Desk model

Downs Surgical present the latest portable units for detection of early fetal life. Both units operate using the ultrasound Doppler principle which enables the heartbeat to be detected as early as 10 weeks gestation. Internal speakers are fitted to both units and headphones are supplied for personal listening.

Downs Surgical

To Downs Surgical Limited,
Church Path, Mitcham, Surrey CR4 3UE, England.
Please send me details of Fetal Pulse Detectors

Name ____________________

Address ____________________

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OG7

IXTH WORLD CONGRESS OF GYNECOLOGY AND OBSTETRICS

FIGO
1979 TOKYO

Date:
October 25-31, 1979
Place:
Hotel New Otani, Tokyo, Japan
Deadline:

Registration
July 31, 1979

For all necessary information and application forms, please write to:

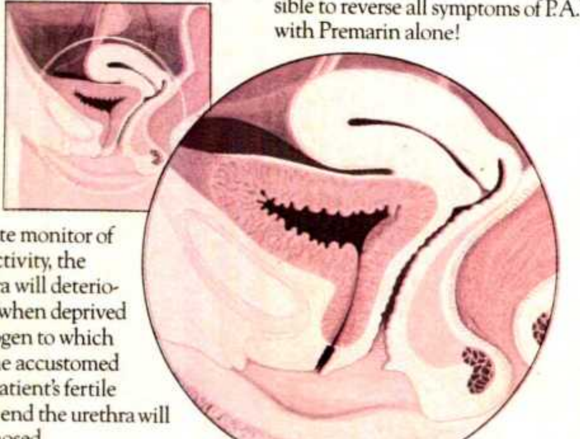
Secretariat;
IXth World Congress of Gynecology and Obstetrics
c/o Simul International, Inc.
Kowa Bldg. No. 9
1-8-10 Akasaka, Minato-ku, Tokyo 107, Japan

The Diagnosis and Treatment of P.A.U.

Post-menopausal Atrophic Urethritis

Older women presenting with frequency, urgency and painful micturition often describe their condition as 'cystitis.' There can hardly be a woman in existence who has not suffered the transient discomfort of a 'simple cystitis' at some time in her early life, but as she reaches middle age, although the symptoms may appear to her as the same, the cause, particularly in the absence of infection, may be atrophic changes in the distal urethra responding to hormonal disparities of the menopause.[1]

Untreated, these atrophic changes can be devastating and demoralising in their effect.[2]



A delicate monitor of oestrogen activity, the distal urethra will deteriorate rapidly when deprived of the oestrogen to which it has become accustomed during the patient's fertile years. In the end the urethra will become stenosed.

Treating P.A.U. with Premarin*

Treatment with Premarin 0.625mg tablets can effect a rapid and complete elimination of symptoms.[3]

1. Oestrogen deprivation can be confirmed by vaginal cytology or assumed in the presence of vaginal atrophy or dryness, or other symptoms of oestrogen lack.

2. Low-dose Premarin (0.625mg) administered cyclically – 21 days on, 7 days off – for 3 months. Urethral epithelium is remarkably sensitive to oestrogens and so will respond to this low dose.

3. Frank urinary tract infection should of course be treated with appropriate antibiotics or urinary anti-infectives.

4. If treatment is instigated in the early stages of this disorder – for instance within the first six/twelve months of symptoms – it may be possible to reverse all symptoms of P.A.U. with Premarin alone!

However in patients with a longer history of frequency and urgency, if oestrogen therapy does not effect a rapid and complete elimination of symptoms then the possibility of a secondary urethral stenosis should be considered.

References:

1. Willington, F. L. The Practitioner. 220, 739, 1978.
2. Smith, P. J. B., Ayerst Medical Library Vol. 2, No 2, 1978.
3. Smith, P. J. B., The Practitioner, 218, 97, 1977.

PREMARIN

(conjugated oestrogens)

Premarin tablets

Uses

Menopausal and post-menopausal oestrogen replacement therapy and allied disorders such as post-menopausal osteoporosis, atrophic vaginitis and post-menopausal atrophic urethritis.

Dosage and Administration

Menopausal and post-menopausal oestrogen deficiency: Premarin 0.625–1.25mg daily is the usual starting dose. Cyclic administration is recommended (three weeks' dosage followed by one week's rest). For maintenance, the lowest effective dose should be used. It is recommended that a physical examination, including endometrial assessment, whenever possible be performed at the time of the initial examination, and the endometrium assessed regularly thereafter at 6–12 month intervals. Break through bleeding is an indication for further investigation.

Contra-Indications: Uterine myomatosis. Endometriosis. Cancer of the breast and uterus. Active hepatic, renal and severe cardiac disease. Undiagnosed genital bleeding. Diabetes. Thyrotoxicosis. Pregnancy. Personal history of thrombosis.

Precautions

In the female – prolonged use of unopposed oestrogen may increase the risk of endometrial carcinoma.

Side Effects

Premarin is unlikely to cause gastro-intestinal symptoms, headaches, breast tenderness, salt and water retention which can occur with other forms of oestrogen therapy.

Product licence numbers

Premarin Tablets 0.625mg. PL 0607/5000.
21 tablet pack £0.80.
Premarin Tablets 1.25mg. PL 0607/5001.
21 tablet pack £1.30.
Premarin Tablets 2.5mg. PL0607/0002.
30 tablets £2.40.

Ayerst International

Full prescribing information:
Ayerst Laboratories Limited,
Invincible Road, Farnborough, Hants GU14 7QH.
Telephone: Farnborough (Hants) (STD 0252) 511981.

*Premarin is a Registered Trade Mark.

Would you like to hear more?

The Dopscope is one of the smallest fetal heart detectors available today. It is a Doppler instrument with four main features: high performance, low acoustic power output, miniature size and low cost.

The Dopscope will detect the fetal heartbeat from the tenth or twelfth week of pregnancy and is very simple to use. It is battery operated, and can therefore be used almost anywhere.

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The Australian and New Zealand JOURNAL OF OBSTETRICS & GYNAECOLOGY

Edited by E. V. MACKAY

Published quarterly under the direction of the Australian and New Zealand Councils of the Royal College of Obstetricians and Gynaecologists and the Arthur Wilson Memorial Foundation.

This journal gives the only coverage of new information from Australia and New Zealand concerning Obstetrics and Gynaecology. The journal is well illustrated.

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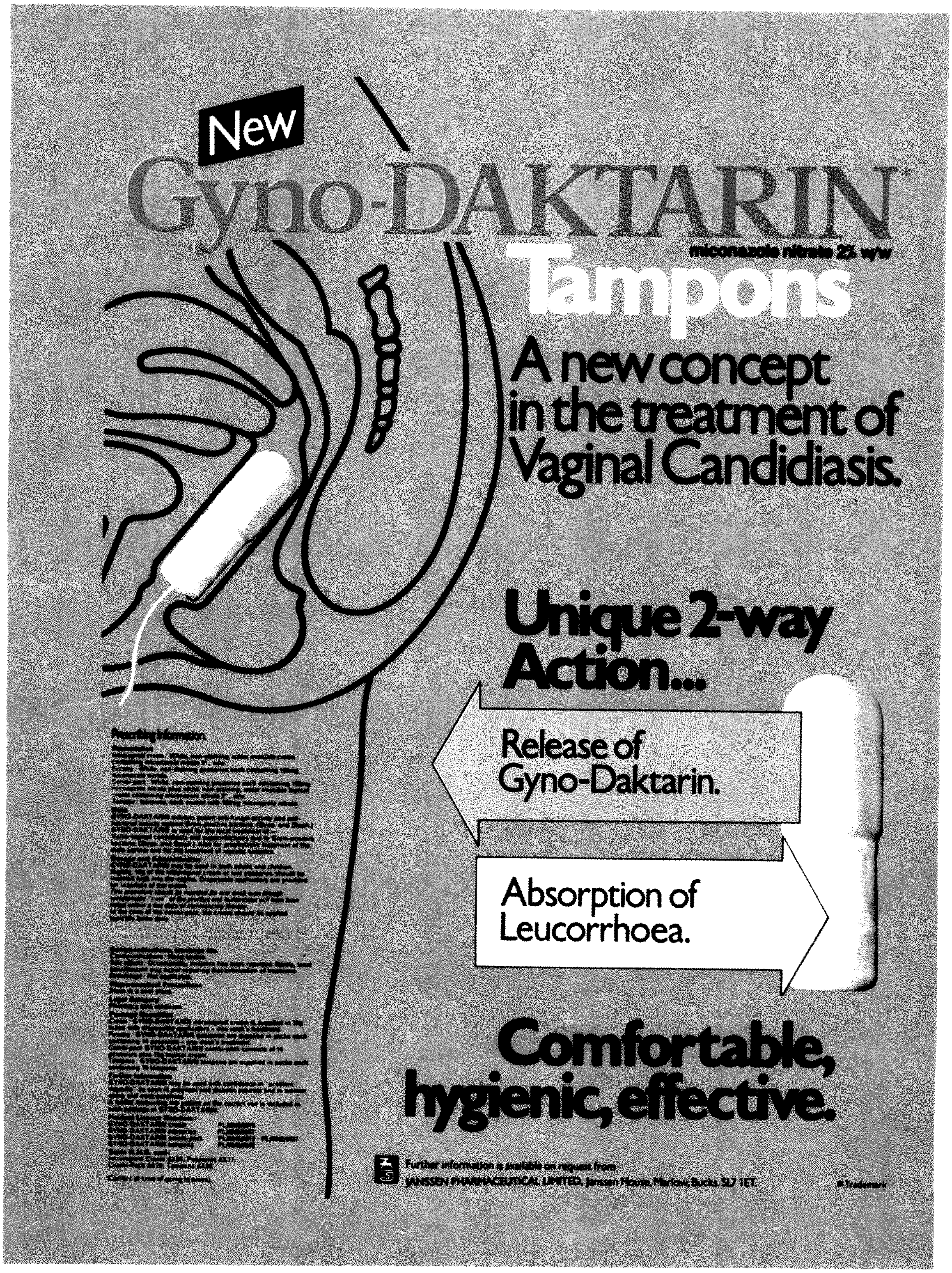

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miconazole nitrate 2% w/w

Tampons

A new concept in the treatment of Vaginal Candidiasis.

Unique 2-way Action...

Release of Gyno-Daktarin.

Absorption of Leucorrhoea.

Comfortable, hygienic, effective.

Prescribing Information.

[illegible]

*Correct at time of going to press.

Further information is available on request from
JANSSEN PHARMACEUTICAL LIMITED, Janssen House, Marlow, Bucks. SL7 1ET.

* Trademark

Growing evidence...widening usefulness

ENDOMETRIOSIS

"...at least as effective as the best 'pseudo-pregnancy' treatment...no initial exacerbations of symptoms, rapid return of normal hormonal function and generally mild side-effects".[1]

BENIGN BREAST DISEASE

"...a significant decrease and elimination of breast pain and tenderness (and of) nodules and lumpy areas..."[2]

MENORRHAGIA

"The efficacy of danazol in the control of heavy menstrual blood loss could prove of value in clinical practice".[3]

GYNAECOMASTIA

"Danazol resulted in a regression of the gynaecomastia in the majority of the present patients studied..."[4]

1. J. int. med. Res. (1977) 5 (Suppl. 3): 86-91.
2. ibid., 101-108. **3.** ibid., 98-100.
4. ibid., 114-123.

Danol

danazol

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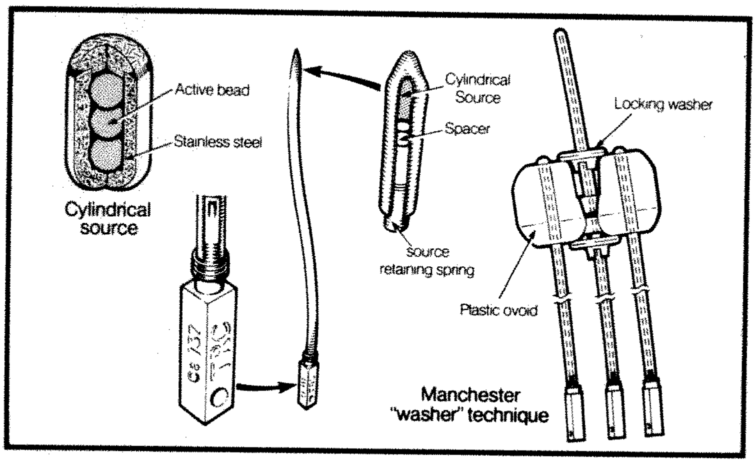

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Prescribing Information

Presentation

The Multiload CU 250 is an intrauterine device made of polyethylene, 3.6 cm in length with 27 cm of copper wire 0.3 mm diameter wrapped around the stem, giving a total surface area of 250 mm² of copper. The flexible side arms ensure that the Multiload CU 250 remains in position as high as is possible against the fundus without the uterine cavity being stretched in any way. The device is preloaded on its inserter and a monofilament nylon thread is attached to the stem.

The Multiload CU 250 can be regarded as a third generation IUD. As a result of its special shape, the Multiload CU 250 represents a breakaway from the principle that IUDs must be firmly embedded into the uterine cavity in order to prevent expulsion. In addition, with 250 mm² of copper wound around the vertical stem, a very low pregnancy rate is obtained without using a maximum surface area.

As a result, the Multiload CU 250 causes practically no cramp and no intermittent bleeding. Contractions of the uterus are cushioned by the wide upper end of the Multiload CU 250, resulting in flexure of the side arms which are designed to prevent expulsion.

The Multiload CU 250 is suitable for both nulliparous and multiparous patients. The insertion procedure is simple and the device can be accurately positioned high up against the fundus. No perforations have been notified to date. Neither an expellor nor sterile gloves are necessary.

Uses

Contraception.

Dosage and Administration

It is recommended that the Multiload CU 250 be replaced every two years.

The recommended procedure for inserting the Multiload CU 250 is contained in the data sheet which is included in every pack, or is available on request.

The ability to become pregnant is normally possible a short time after removal of the Multiload CU 250.

In the case of pregnancy, the Multiload CU 250 must be removed.

Contra-indications, Warnings, etc.

Contra-indications: pregnancy, congenital or acquired abnormalities of the uterine cavity; pelvic inflammatory disease, either pre-existing or in a patient with a history of such conditions; acute cervicitis, acute or sub-acute salpingitis; endometritis or infected abortion in the past three months; dysfunctional uterine bleeding; cervical dysplasia; suspected or proven carcinoma of the cervix or endometrium; uterine polyps or fibroids; disturbance of the blood clotting mechanism and known allergy to copper.

Some women are troubled by abdominal cramps after insertion. Post insertion syncope may occur in some nulliparous patients. Intermittent bleeding or prolongation of menstruation is not usual during the first two or three cycles.

Package quantities

1 outer package containing 10 Multiload CU 250

10 inner packs each containing 1 Multiload CU 250 ready for immediate use and fitted with a disposable inserter.

Product Licence Number 0065/0062

Basic Cost 1 Multiload CU 250
£5.00.

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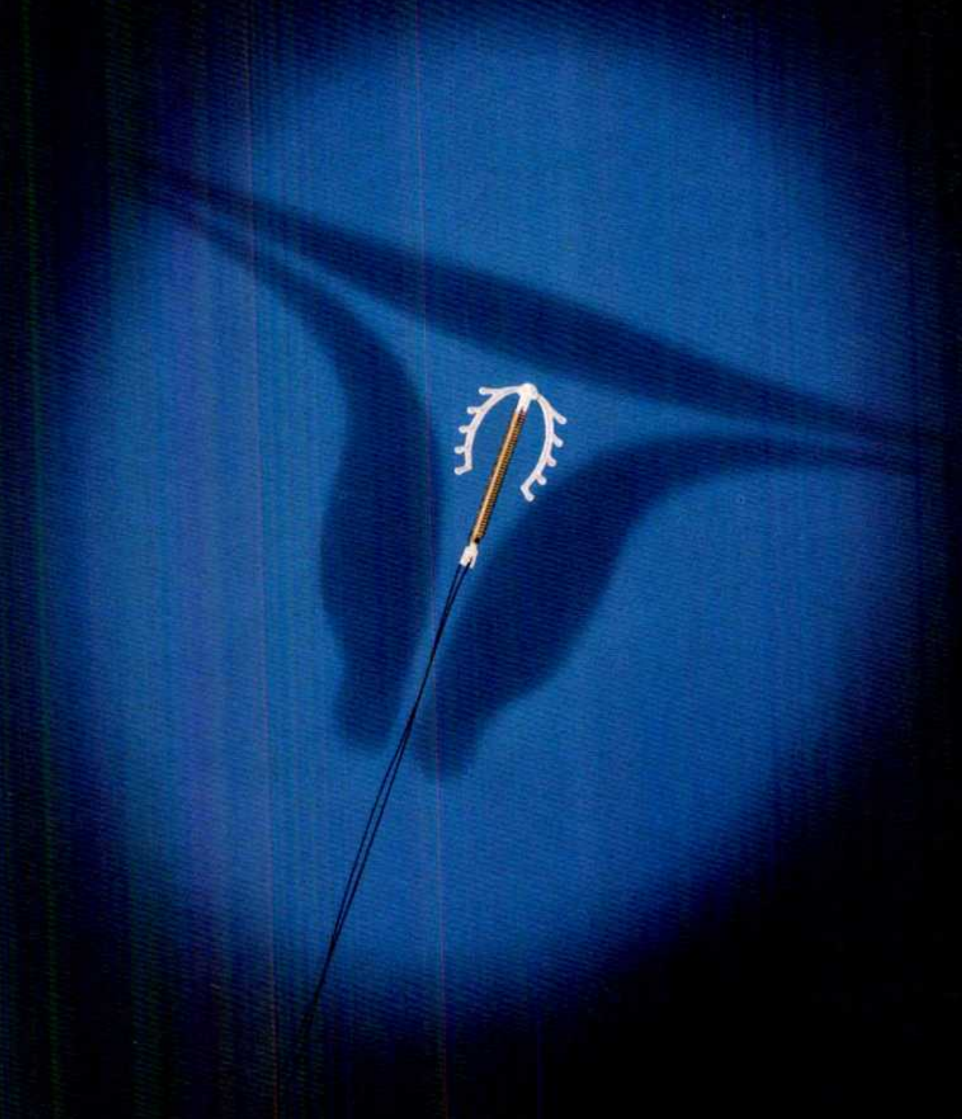

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Organon References and further information are available on request
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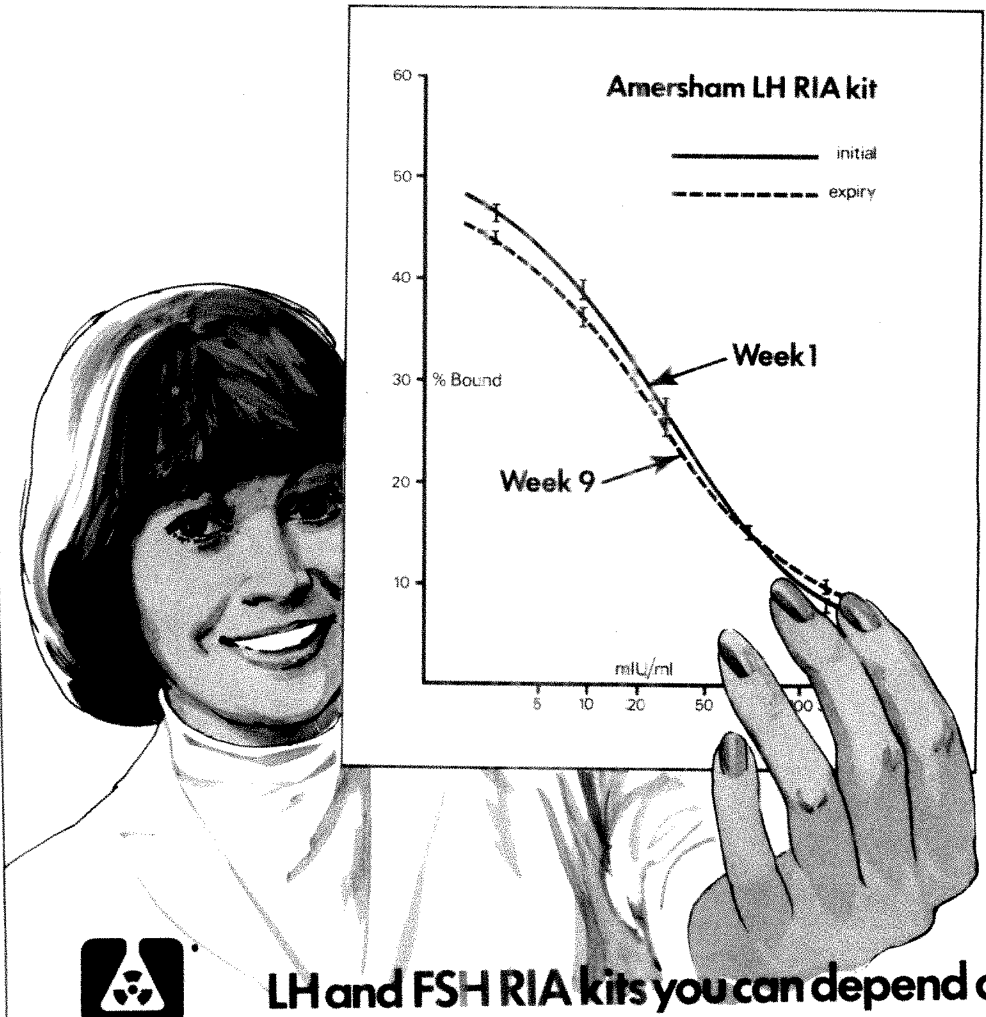

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A Canesten Duopak is available containing vaginal tablets for the patient and cream to treat her vulvitis or her partner to help prevent reinfection.

Canesten® clears vaginal candidiasis in three days

clotrimazole

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1. *Current Medical Research and Opinion*, **3**, 83–88, 1975. 2. *British Journal of Venereal Diseases*, **53**, 126–128, 1977. Canesten is available as vaginal tablets, each containing 0.1g clotrimazole, in packs of six with applicator. Canesten Duopak contains six vaginal tablets with applicator plus 20g cream (1% clotrimazole). Dosage – vaginal tablets: Two vaginal tablets should be inserted daily for three consecutive days. Alternatively, one vaginal tablet daily for six days. Duopak: Vaginal tablets as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. Side effects: Rarely patients may experience transient local mild burning or irritation. N.H.S. costs: 6 vaginal tablets with applicator £1.92; Duopak £3.13. Product Licence

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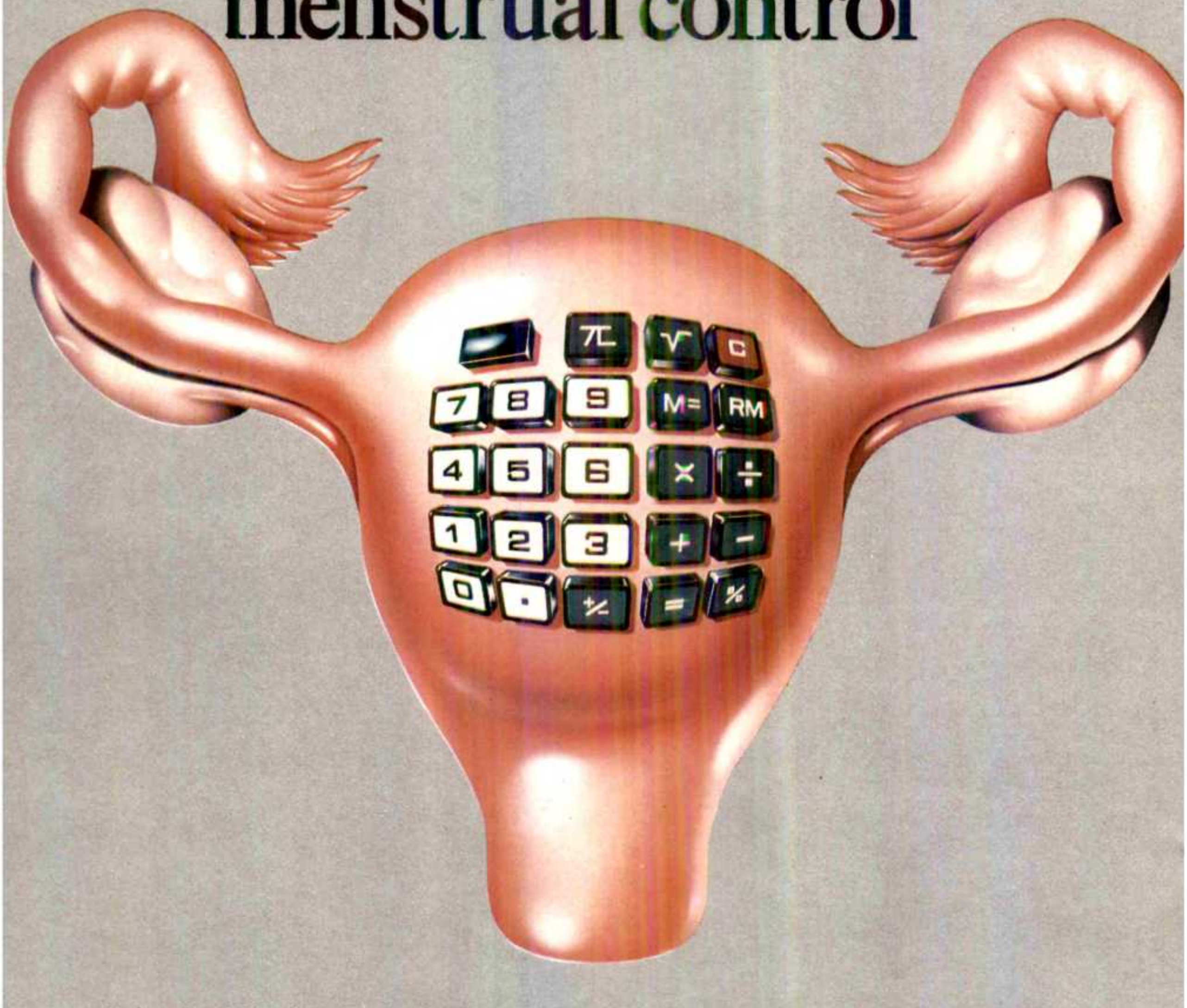

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British Journal of Obstetrics
and Gynaecology

Vol 86 No. 6 NEW SERIES JUNE 1979

ANOTHER EMINENT VICTORIAN*

BY

SIR STANLEY CLAYTON, MD, MS, FRCP, FRCS, FRCOG

Professor Emeritus, University of London

FOR a retired obstetrician from far south of the Border to be invited to deliver the Simpson Oration, particularly on a St Andrew's day, is a signal honour which I very deeply appreciate. My remarks will be to some extent outward looking, as I often found myself trying to discover how James Young Simpson saw the world rather than how the world saw him.

He was born on 7th June, 1811, and he died on 6th May, 1870. He was the eighth and youngest child of Mary Jarvey (Jervais) of Huguenot descent, and of David Simpson, a baker in Bathgate, a small place about 19 miles from Edinburgh. Into what sort of world was he born?

George III was still king at far off Windsor, but now as a lonely and demented prisoner in the harsh care of his doctors. During the old king's reign there had been many more years of war than of peace; the Seven Years War had been followed by the American Rebellion and that by the French Revolution. Although Nelson had given his life to end the threat of invasion by Buonaparte, it was not until Simpson was nearly four years old that victory was finally secured at Waterloo, where Scottish regiments gave distinguished service. For the rest of Simpson's life there was no further threat to the security of these islands and this long peace at home "was one cause of that . . . belief in 'progress' as a law of history that cheered the Victorian mind" (Trevelyan). The sordid reign of George IV, only a little alleviated by his good taste in painting and literature, and the short reign of William IV, passed while Simpson was learning to be an obstetrician, and in maturity he is to be thought of as another of the 'Eminent Victorians'; although I hope to speak more kindly than Lytton Strachey wrote.

In these years the population of Scotland was growing fast. This was a real increase; emigration of dispossessed crofters from the Highlands was balanced by immigration of starving Irish labourers during the potato famine. The birth rate rose and there was, at least after infancy, a longer expectation of life; but many workers were condemned to spend their lives in slum tenements, and there was an enormous separation between the poverty of the workers in factories and mines and the comfort of their employers and the professional classes. Young children worked long hours in the mills, and we read of a woman of Edinburgh who made shirts: "She was paid 4½ pence for each garment, which after deducting thread and light, left her 2 pence for eight hours work: so, for a bare subsistence she had to work 18 hours a day". The Corn Laws drove up the price of bread, and the bitterness of the working people was increased because they were excluded from the franchise.

Simpson's life lay in a time of progress and social improvement, with electoral reform and new humane legislation. The efforts of Lord Ashley, of writers such as J. S. Mill, Kingsley and Dickens, of Robert Owen the radical millowner, and of the working man Francis Place, combined to bring in the Factory Acts that began a progressive revolution which has continued to this day. Concurrently the scientific experiments of Faraday and Joule led to the equations of Kelvin and Clerk Maxwell; while the tunnels, bridges and ships of the Brunels were outward signs of Victorian progress.

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* The 18th J. Y. Simpson Oration given at the Royal College of Obstetricians and Gynaecologists on 30th November, 1978.

Support from his family for the favourite youngest son allowed James to enter Edinburgh University at the age of 14 for courses in Latin, Greek, mathematics and philosophy. His Latin exercises were usually marked 'bene' but, characteristically, when they were returned he used to list the corrections which seemed to him just and then write boldly 'I object to the rest'. He never finished these courses because he happened to lodge, at a rent of three shillings a week, with a medical student from Bathgate whose books he found preferable to his own. At the age of 16 Simpson changed to the Faculty of Medicine, and he gained the Diploma of the College of Surgeons before he was 19.

It is perhaps curious that he became an obstetrician, as midwifery was then described as "the lowest and most ignoble of the medical arts". He greatly admired Robert Liston the surgeon, but it may be that the horror of surgery without anaesthesia was too much for him, and it is known that on one occasion he fled from the theatre. His MD thesis, written when he was 21, and in Latin as was then required, was on the pathology of the placenta, and it so impressed the Professor, John Thomson, that he invited Simpson to become his assistant at an annual salary of £50, a scale of remuneration that the impecunious young man was glad to accept. Thomson advised Simpson to take up obstetrics, and he was also influenced by the Professor of Midwifery, James Hamilton, a little fat man with an ill-fitting wig, who was described as the "most contentious of a singularly pugnacious professoriate".

To prepare himself for his specialty Simpson made a Grand Tour. After visiting hospitals in England he crossed the Channel—for a fare of one guinea—to travel in France and the new Kingdom of Belgium. While returning through Liverpool he visited his cousin Walter Grindley, whose daughter Jessie captured his heart. Home again in Edinburgh he served as part-time House Surgeon at the Lying-in Hospital, before becoming an extramural lecturer. Compared with the modern programme of higher training (which is in my opinion far too long) Simpson's formal training seems perfunctory, but at the age of 28 he applied for the University Chair in succession to Hamilton. The application of the baker's son was opposed by most of the Faculty because of his youth, his alleged inexperience and his unsuitability for female practice as he was unmarried. In the nick of time James married Jessie, and he reinforced his application with 59 printed testimonials, including one from his students who wrote of "his unceasing attention to their interests . . . in which he spared neither time nor labour". The hotel keepers of Edinburgh did good business in accommodating patients who came from a distance to consult the doctors of the capital, and one member of the Town Council, which was responsible for the appointment, feared that the young man would bring little trade—how wrong he proved to be. In the event Simpson was elected by 17 votes to 16.

The students found that Simpson took immense pains in preparing his lectures. As his practice took up most of the daylight hours he "rose regularly at 3 every morning to get all ready by breakfast" and before each lecture he wrote notes for the day on the blackboard. He was reported to have a "ready and fluent manner" and "a wonderful facility for imparting information". The mockery of students is often based on affection. Figure 1 shows a student's view of the Professor invigilating. He was then aged 44, but I do not believe that he was as fat as he is shown. The students appear as scruffy as those of today, but each has brought his top hat, perhaps a symbol like the best tie which the modern student wears for a viva. There are no women students, and no fountain pens. Think of the scratchy labour of writing an examination paper with a quill. The Professor has no coffee, but he seems to have a snuffbox and a cigar box, and what looks like a doughnut.

Previous orators and many anaesthetists have described Simpson's introduction of chloroform into anaesthesia, and I need say little about that. Nor do I wish to spend any time on the quarrels about priority between the Americans Long, Jackson, Wells and Morton; Simpson gave unreserved credit to those who had used laughing

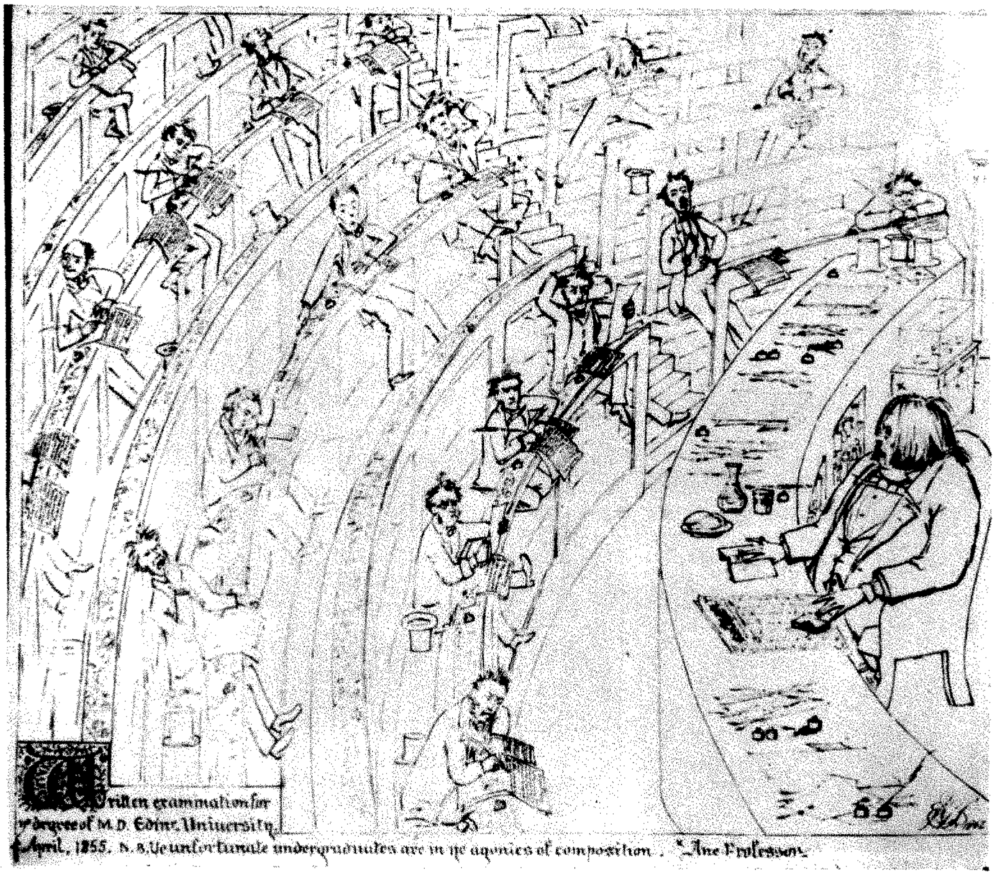
Written examination for ye degree of M.D. Edinr. University, April, 1855. N.B. Ye unfortunate undergraduates are in ye agonies of composition. *The Professor.

FIG. 1

A student's caricature of Professor Simpson invigilating.

(*By courtesy of the Wellcome Trustees*).

gas or sulphuric ether before him. He wrote little about the use of chloroform in surgery, no doubt because there was then very little operative gynaecology. In his 'Clinical Lectures' he described Sims' operation for vesico-vaginal fistula, an operation like that of Le Fort for prolapse, and dilatation of the cervix for dysmenorrhoea. For cervical cancer the only treatment was amputation or cauterization of the cervix. Fibroids were excised if they were polypoid; otherwise they were treated with the galvanic needle—with what results is not stated. Apart from tapping, the only gynaecological abdominal operation that he described was ovariotomy, but the details appear to have been taken from Spencer Wells. Caesarean section was only recommended when the obstetric conjugate was less than $1\frac{3}{4}$ inches.

On the other hand he wrote at length about the use of chloroform for obstetrics. He fought a valiant battle with many clergy and not a few doctors who declared that pain in childbirth was both natural and salutory, and ordained in Scripture. He attacked the divines on their own ground, pointing out that the Hebrew word 'etzebh' in Genesis means toil or labour rather than pain, and that when Adam had a rib excised the Lord God caused a deep sleep to fall on him. In spite of such opposition the speed with which chloroform came into general use was remarkable. Simpson's first publication was in November 1847; less than a year later he had used it during labour for 150 cases in his own practice. It gave him special satisfaction that Dr Protheroe Smith of London mentioned 125 cases at this time because "The London physicians have, on several occasions, specially distinguished themselves by their determined and prejudiced opposition to all innovations in practice not originating among themselves". It is well known that the administration of chloroform to Queen Victoria at the birth of Prince Leopold in 1853 gave the royal imprimatur to obsteric analgesia, and it may have been Prince Albert who persuaded her to try it. Simpson met the Prince in 1848 and wrote afterwards that they discussed Scotland, chloroform and the magazine 'Punch' which, with its comments on affairs, was then a new social phenomenon.

Eminent Victorians not only expected progress—they wished to take part in it. Amazing advances in communication came with the railways and their accompanying telegraph wires. During Simpson's childhood a journey from Edinburgh to London on the mail coaches involved more than 48 hours of atrocious discomfort. When he was 14 years old the first steam-hauled passenger service began between Stockton and Darlington, but only 25 years later regular trains were running to Scotland on both the East and West coast routes. As Trevelyan wrote "The new railway system opened out the Highlands of Scotland . . . The moorland expeditions of the Queen and Prince Albert from Balmoral, and the deer which the Prince shot and Landseer painted, popularized Highland scenery with all classes of the English"—and everything Scottish suddenly became fashionable. Mail was carried on the trains two years before the penny post came in, and better communication allowed medical knowledge to be more widely spread and generally shared.

Dr Simpson's practice became very large, and his house at 52 Queen Street became crowded with patients. Soon he bought a carriage, painted claret and lined in red, in which he delighted, and he engaged a butler to manage the waiting throng. It was reported that "patients arriving together draw lots for priority, have tickets, and come as called".

Contributions of Simpson to the medical thought of his time that have perhaps not been given sufficient weight by previous orators are those relating to puerperal fever and infection after surgical operations, and I particularly refer to observations which he made to the Medical and Chirurgical Society of Edinburgh in 1851. When he spoke, Pasteur and Cohn had not yet shown that fermentation and putrefaction were the work of minute living organisms. Although Dolland had introduced the oil-immersion lens for microscopy seven years previously, Abbe's substage condenser was not available, and it would have been nearly impossible to recognize most pathogenic microbes; their systematic study had to wait for Weigert's stains and Koch's culture media.

At this meeting Dr Arneth from Vienna, a colleague of Semmelweis, gave (in Simpson's words) "incontrovertible evidence of puerperal fever being propagated . . . by medical men carrying on their fingers matter capable of producing it from bodies which they were dissecting, and inadvertently inoculating the matter into . . . the vagina of patients in labour". Simpson mentioned several cases in support of this: "in 1840 upwards of 400 women were delivered by different midwives in connection with the Lying-in Hospital in Manchester. These 400 women were delivered in different parts of the town at their own houses. Sixteen of them died of puerperal fever . . . this could not have arisen from any general epidemic, or atmospheric or telluric influence; for the fatal cases occurred in no particular district, but were scattered through different parts of the town. Now these 400 women . . . were attended in their confinements by twelve different midwives. Eleven of the twelve midwives had no puerperal fever among their patients. The sixteen cases had occurred in the practice of only one of the twelve". Simpson also said: "Mr Sidey of this city had a rapid succession of five or six cases of puerperal fever in his practice, at a time when it was not known to exist in the practice of any other practitioner in this locality. Dr Simpson, who had then no full or proper belief in the contagious propagation of puerperal fever, attended the dissection of two of Dr Sidey's patients and freely handled the diseased parts. The next four cases of midwifery that Dr Simpson attended were all affected by puerperal fever, and it was the first time he had seen it in his practice".

Where Simpson advanced further than Semmelweis was that he stated that puerperal fever could occur if the medical attendants had been in contact with cases of erysipelas, surgical pyaemia or gangrene, and he noted that medical men had died of erysipelas after receiving skin punctures at autopsies of patients who had died of puerperal fever. He seems to have thought that this cross-infection (to use a modern term) could also occur from inhalation. In a later paper (1867) he wrote "Pasteur . . . and others maintain that the atmosphere is full . . . of living spores and germs of various infusoria etc, which, when they find a proper nidus, lead on to fermentations, putrefaction, suppurations etc". This is the only evidence I have found that Simpson recognized Pasteur's work.

I do not know whether he was familiar with the observations of White of Manchester (1763), of Gordon of Aberdeen (1795) or even of Oliver Wendell Holmes (1843) when he gave these papers. He knew that Semmelweis believed that putrefying animal matter constituted what was then called the virus, but Simpson did not accept that the virus was the result of putrefaction. He stated that it was an 'inflammatory secretion'. By this he did not mean a living and parasitic organism but (as we might now say) a toxin, which he believed was specific for each disease. Today it is easy for anyone to believe in infection by bacteria as they can be seen growing in a culture or a neatly stained preparation; Simpson had only clinical observations on which to base his ideas.

He certainly realized that his ideas about the spread of puerperal fever also applied to surgical infection; he wrote "no doubt under similar circumstances surgeons could . . . inoculate into the wounds which they made or dressed similar matter producing the similar disease of surgical fever". "He had no doubt that it would take many long years fully to convince surgeons of this fact; but still it was his conviction that surgeons would ultimately both believe and act upon it, and that their doing so would be a means of preventing many of the numerous deaths which now occur after operations". These were prophetic words.

Sixteen years later (1867) Simpson attended the Annual Meeting of the British Medical Association in Dublin, where a young surgical colleague, aged 29, from the Royal Infirmary in Edinburgh, spoke about his work. It is possible, but difficult, to believe that Simpson failed to understand that Lister was describing a revolutionary new surgical principle that did not depend on any particular chemical substance such as carbolic acid, but it must be to Simpson's discredit that he attacked Lister with venom, writing to 'The Lancet' to say that carbolic acid had already been used by Continental surgeons as a dressing for infected wounds without benefit. It is fair to remember that Simpson tried a great variety of substances while he was looking for a better anaesthetic than ether, and he may have thought that Lister was merely seeking a better chemical substance to apply as a dressing—but I fear that he was influenced by emotion rather than reason. Lister had been the assistant and was now the son-in-law of James Syme, the Professor of Surgery. Simpson and Syme were contrary in all things; that Lister was Syme's protégé was sufficient reason for Simpson to condemn him.

In the year before Simpson's death Lister succeeded Syme in the Chair of Surgery at Edinburgh, and it was only after that time that the details of the antiseptic system, with all the paraphernalia of the carbolic spray, antiseptic gauze dressings and sterilized catgut ligatures were fully developed. Simpson therefore never had a chance of seeing the results of antiseptic surgery for himself.

It must not be believed that Simpson was not deeply concerned about the appalling mortality of surgical operations in his time. In the same year that Lister published his first paper on the antiseptic system (1867) Simpson gave his Presidential Address to the Association for the Promotion of Social Science. For about twenty years before that time he had been writing about the dangers of 'hospitalism'. He compared the results of amputations of the limbs in large hospitals with those in smaller hospitals, especially those in rural or provincial practice. Among 2089 patients undergoing amputations in the large hospitals there was a mortality of 41 per cent; whereas among almost exactly the same number of patients in country practice the mortality was 11 per cent. From details in his report it can be discovered that in the large hospitals pyaemia, gangrene, secondary haemorrhage, and what was called exhaustion and was presumably the result of sepsis accounted for 87 per cent of the deaths, but in the country hospitals for only 35 per cent. Simpson concluded that there was danger in aggregation of patients, and he wished every sickroom to have "a great surplus of air" which "by diluting and carrying away the morbid substances evolved from the bodies and excretions of the sick" would reduce the risk to a minimum.

Before Lister introduced carbolized catgut Simpson had recognized the danger of unabsorbable but porous ligatures which only came away when the arterial tissues softened by suppuration, and he devised a method of compressing arteries by passing metal pins beneath them which could be withdrawn as soon as thrombosis had taken place. Although this method of 'acupressure' was soon superseded by the use of absorbable and aseptic ligatures, Simpson's continued advocacy of it was another reason for his opposition to Lister, particularly after Syme tore up a copy of his paper in front of the students and threw it into the box of blood-stained sawdust in the operating theatre.

Simpson had much mechanical skill, and Mr J. A. Chalmers has admirably described his invention of a vacuum extractor. In a letter to a friend Simpson characteristically wrote that he had been up three nights working on the instrument. He set up a forge in his cellar, the noise and smoke of which led his cook to give in her notice. The extractor was used on a few patients, but in the end he seems to have preferred the obstetric forceps which he modified to his own pattern.

His energy was amost unlimited. In her excellent biography Myrtle Simpson (1972) describes how, after he had travelled to Oxford to receive a Doctorate of Civil Law, he met his son Wattie (Walter) at Devizes. "They swallowed a hasty lunch, then hired a carriage to drive to Avebury to see the stone circle, returning at 11 p.m. By 6 next morning they were on their way to Stonehenge, then back to Devizes before catching a train to Bath. Arrived there in the evening, they immediately hired a carriage to drive to some other standing stones some miles from Bath. On getting back to the hotel at 11 p.m. Simpson found a telegram calling him to a case in Northumberland. He lay down for an hour or two, then caught the 3 a.m. train to London. He took a quick breakfast in London and then continued directly to Northumberland. After seeing his patient he filled in the time before the Edinburgh train by visiting a Roman fort. He eventually reached home, full of enthusiasm, at 2 a.m. Wattie had meanwhile retired to bed from exhaustion".

What was the outward appearance of this extraordinary restless extrovert? Dr Channing, a visitor from Boston in New England, wrote "The Professor is well made for dispatch. He is small, stout, with small feet, and his step is short and quick . . . I have almost to run somewhat not to lose him . . . His head is large, covered with a

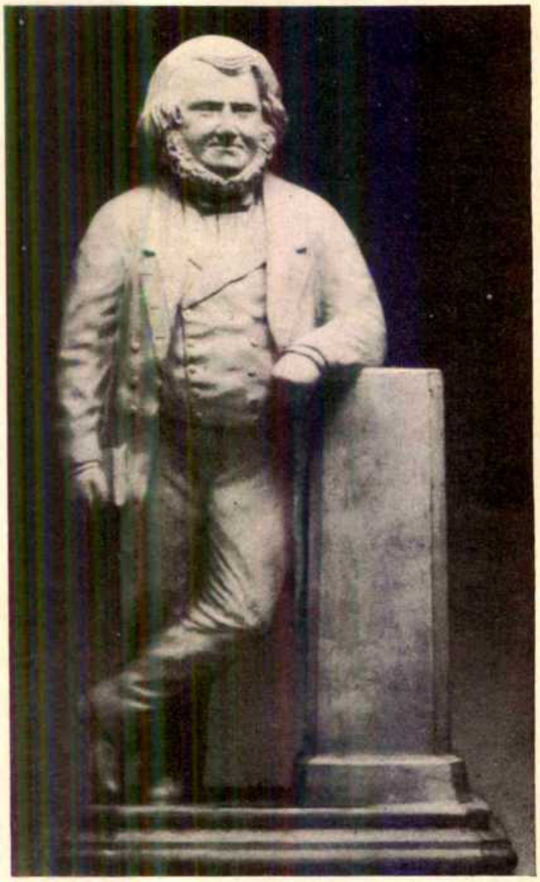

FIG. 2

Statuette of Simpson presented to the library of the Royal College of Obstetricians and Gynaecologists by Sir Bernard Dawson.

(Photograph kindly supplied by Mr Michael Fogarty).

profusion of black hair . . . His dress is always black, with a remarkably nicely arranged white neckcloth". The statuette from the library of the Royal College of Obstetricians and Gynaecologists (Fig. 2) shows his figure well.

While he had an attractive and sympathetic personality, was both admired and loved by his patients, was devoted to his children and also to a dreadful succession of dogs which attended his consultations, was generous and careless with money—yet he could be extremely contentious. Apart from a lifetime of opposition to Syme, he fought a series of battles, apparently almost with enjoyment, with superintendents of hospitals, with University authorities, with his former assistant Keith and several other colleagues—on one occasion needing to be restrained from entering upon a duel.

His curiosity was insatiable. He was interested in the new art of photography and in astronomy, and he was sufficiently expert in archaeology to be named Professor of the subject by the Royal Scottish Academy. Consideration of such practical matters as the manufacture of rum in Tobago, the shipping of guano from the Hebrides, distillation of oil from Irish peat, preservation of meat and the notification of smallpox was mixed with speculations on clairvoyance, together with the writing of very bad verse. With such a torrent of ideas it is not surprising that he was sometimes wrong, as in his teaching that placenta praevia was best treated by extraction of the placenta vaginally before the child.

Simpson's compassion for the poor involved him in a variety of schemes. He subjected the Member of Parliament to a "midnight ramble down the High Street, in and out of the wynds between the towering dark lands to see the windowless and overcrowded rooms, and to smell the appalling drains"—or lack of them. At various times he signed a petition against slavery; canvassed for electoral reform; spoke against the Crimean war and gave 1000 doses of chloroform to Miss Nightingale; entered the dispute about the disruption of the Church of Scotland; organized a petition for drainage of the city marsh; ridiculed homoeopathy; supported the entry of women into medicine; and played a crucial part in the negotiations for the Medical Act of 1858.

He was also influenced by the traditions of the Scottish Kirk. Like so many other Eminent Victorians he had to face the conflict between the literal interpretation of the Bible and the conclusions of Lyell, Wallace and Darwin. And family tragedies influenced his beliefs. Because of the lack of effective treatment for infections and the limitations of surgery few Victorian families were without experience of the loss of children. After the loss of his first child Maggie from an infection of the throat after measles and the loss of his son Jamie at the age of 15 he sought help from religion, and became an active member of the Carrubers Close Mission. Myrtle Simpson has described how curious souls attended the meetings to see how Simpson looked as a Christian; they found him to be Simpson still, full of zeal but firmly rejecting the belief that the world was suddenly created six days before the appearance of Adam. It is sad that his last days were clouded by the loss of two other children, his doctor son David at the age of 24 from appendicitis, for which there was then no surgical treatment, and his daughter Jessie aged 17, only a month later.

In 1866 James Young Simpson was made a Baronet, and in the year before his death he received the Freedom of Edinburgh. At this time he was troubled with angina, but worked to the end, even having a few patients brought to his bedside.

I may end by asking what field Simpson would have chosen if he had the scientific opportunities of today. With his developed social sense I am sure he would have welcomed the progress we have made in family planning. Our present interest in the fetus would have excited him. One passage in his "Obstetric Works" is of great interest. The stethoscope had been available since 1816, and during his lifetime became used to detect fetal heart sounds. Simpson wrote "The death of the child was most frequently threatened when the fetal pulse became slower and slower . . . This was apparently the way in which the foetus generally perished during slow and protracted labours—the aeration of the blood by the placenta being thus rendered imperfect . . . There were, however, rarer cases in which danger was indicated to the child by the foetal pulse becoming more rapid, reaching 150 or 160 beats in the minute, and at the same time becoming very irregular or intermittent". What

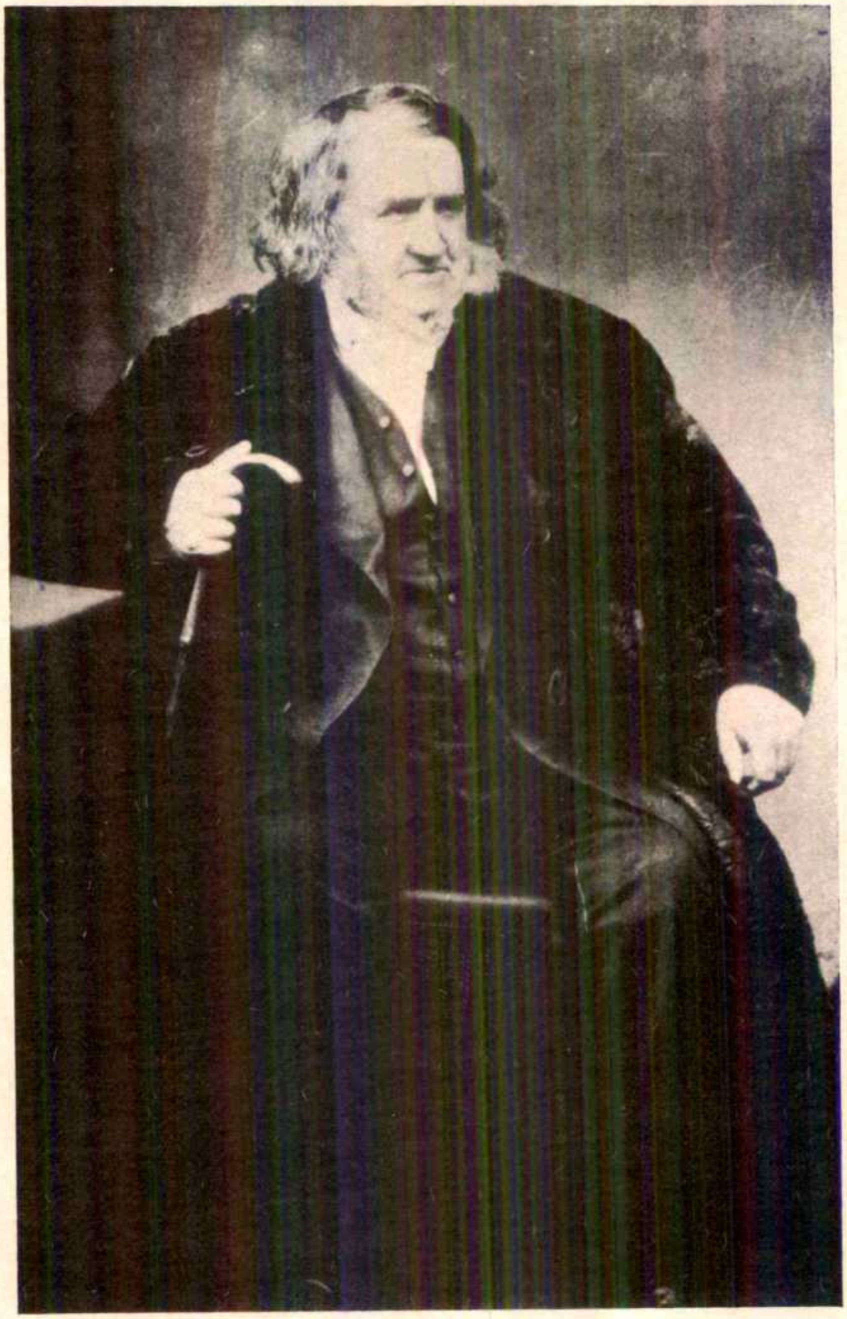

FIG. 3

Sir James Young Simpson, Baronet.

(By courtesy of the Wellcome Trustees).

excellent use he would have made of the fetal ratemeter and fetal blood sampling.

In 1840 a contemporary of Simpson, Thomas Carlyle, delivered his lectures "On Heroes, Hero-worship and the Heroic in History" and said "The history of what man has accomplished in this world is at bottom the History of the Great Men who have worked here . . . Great Men, taken up in any way, are profitable company. We cannot look, however imperfectly, upon a great man, without gaining something by him".

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PLACENTAL INTERVILLOUS BLOOD FLOW MEASURED BY INHALED ^{133}Xe CLEARANCE IN RELATION TO INDUCTION OF EPIDURAL ANALGESIA

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Summary

A method of determining placental intervillous blood flow (IBF) using an inhaled ^{133}Xe technique is described. The method was used to investigate the effect on IBF of epidural analgesia with an initial dose of 37·5 mg bupivacaine. There was no statistically significant reduction in IBF following the initial dose of bupivacaine in spite of a small but statistically significant reduction in mean blood pressure. There was no significant correlation between the change in mean blood pressure and the percentage change in IBF following epidural block although the single largest percentage reduction in IBF was associated with the largest fall in mean blood pressure.

SYMPATHETIC blockade associated with lumbar epidural analgesia, even with subjects in the left lateral position to prevent aorto-caval compression, leads to changes in the distribution of maternal regional blood flow (Weaver *et al*, 1975). This could lead to redistribution of organ blood flow and, in particular, a reduction in placental intervillous blood flow (IBF). Brotanek *et al* (1972) reported indirect qualitative measurements of uterine blood flow using a heated thermistor inserted into the cervix of women having epidural analgesia in labour. They observed a temporary decrease in uterine blood flow coinciding with systemic hypotension; a decrease in uterine blood flow did not occur without simultaneous hypotension.

Direct quantitative measurement of intervillous and myometrial blood flow calculated from the clearance of intravenously injected ^{133}Xe is feasible in women with anterior placentae (Rekonen *et al*, 1976). Using this technique Joupilla *et al* (1978*a*) studied the effect on IBF of lumbar epidural analgesia for Caesarean section using 16 to 20 ml of 1·5 per cent lignocaine with adrenalin 5 μg per ml. Impairment of IBF after epidural block was observed in 7 of the 9 patients studied but the mean decrease (13 per cent from the control value) was not statistically significant. The most notable decrease in IBF occurred in two patients with simultaneous arterial hypotension. In another study Joupilla *et al* (1978*b*) measured IBF using the same ^{133}Xe technique in the first stage of normal labour, before and after segmental epidural analgesia with a small dose (20 mg) of 0·5 per cent bupivacaine with or without 20 μg adrenalin.

There was no significant change in IBF 15 to 20 minutes after epidural block compared with control values.

The initial dose of bupivacaine given for lumbar epidural analgesia in labour at Northwick Park Hospital and in most other maternity units is usually between 20 and 50 mg (8 ml of 0·25 per cent to 10 ml of 0·5 per cent solution). We measured placental IBF in relation to a standard initial dose of 37·5 mg bupivacaine without adrenalin (10 ml of 0·375 per cent solution) in 20 patients scheduled for induction of labour, using an inhalation ^{133}Xe technique.

Patients and Methods

The 20 patients selected were scheduled for induction of labour between 38 and 41 weeks gestation, had requested epidural analgesia, and were known to have anteriorly situated placentae from previous ultrasonography. The use, as described, of ^{133}Xe for measuring IBF in volunteers was approved by the Department of Health and Social Security Isotope Advisory Panel. Informed consent to the procedure was obtained from each patient in the manner laid down by the Northwick Park Hospital Ethical Committee. IBF was measured before and again 30 minutes after an initial dose of 37·5 mg bupivacaine without adrenalin administered via an epidural catheter inserted through the L2–3 or L3–4 interspace. In 13 of the patients two consecutive measurements of IBF were made before epidural block to establish the reproducibility of the method. In two patients the post-epidural measurements were excluded because of recording errors (both of these patients had had two control measurements before epidural block which were not subject to recording error and which are included in the analysis of reproducibility). After completion of the post-epidural placental blood flow measurement, labour was induced by artificial rupture of the membranes, taking advantage of the existing analgesia, followed by oxytocin infusion.

The position of the placenta, located by ultrasound B-scan, was marked on the anterior abdominal wall. After placement of the epidural catheter the patient was positioned comfortably recumbent, supported with pillows to provide at least 20 degrees of left lateral tilt. This position was maintained continuously throughout the pre-epidural measurements, the administration of local anaesthetic solution via the epidural catheter and the post-epidural measurement. A slow intravenous infusion of five per cent dextrose in water was maintained throughout.

The dermatome distribution of analgesia was assessed immediately before post-epidural IBF measurements by loss of painful sensation to pinprick. The range of analgesia was T11–L5 to T9–S5.

Routine for IBF measurements

Two low energy gamma ray detectors (ERD Engineering), with 3 × 38 mm sodium iodide crystals and 5 cm internal diameter parallel collimators 6 cm long, were placed close to the body surface, one over the centre of the placenta and one over a region of the uterus outside the placental limits.

A breathing circuit, attached to an aviator's face mask, allowed inspiration from the atmosphere via an inlet valve and from a reservoir bag containing 2 mCi ^{133}Xe in 2 litres oxygen, controlled by a tap. With the tap open the patient rebreathed expired gases and with the tap closed atmospheric air entered the mask via the inlet valve. Expired air was channelled to a lead-shielded Douglas bag. A third gamma ray detector was used to measure activity in the expired air by drawing air through a cuvette mounted close to the thin sodium iodide crystal (Crawley and Veall, 1975). The breathing procedure was rehearsed without ^{133}Xe to allow the patient to become accustomed to the face mask and breathing instructions. Thereafter the patient was asked to breathe out, the tap was opened to the reservoir bag and the subject took three slow breaths over 25 to 30 seconds. When the reservoir bag had emptied for the third time the tap was closed and the mask was held in place for a further two to three minutes until the peaks of the expired air recording were less than one per cent of the height of the initial peak. Expired air counts were recorded digitally with a 0·8 second dwell time and, via a ratemeter, on a chart recorder. The curve described consisted of a series of peaks of decreasing amplitude. The initial peak count rate was approximately 5000 counts per second with the single channel analyser set to accept all energies from 20 keV to 120 keV.

The outputs from the detectors over the abdomen were separated by single channel pulse height analysers to give separate recordings for the 30 keV and 80 keV peaks of ^{133}Xe, recorded digitally with a dwell time of 10 seconds and on chart recorders. Recording was continued for 25 minutes. The peak counts in each channel were approximately 150/second over the placenta and 50/second away from the placenta.

A curve drawn through the peaks of the expired air recording was deconvoluted from smoothed curves representing the sum of the 30 keV and 80 keV peaks (Veall, 1971; Simon, 1972) using an algorithm that is economical of computer time but requires a continuously falling input curve (Reeve and Crawley, 1974).

The deconvoluted curve was resolved into two components, the faster representing the placental clearance and the slower the myometrial clearance.

IBF was calculated from the half-period, $t_{\frac{1}{2}}$, of the fast component of the bi-exponential curve assuming that exchange of ^{133}Xe between intervillous maternal blood and the fetal circulation is negligible. The intervillous flow, f, in ml/100 ml/minute was given by

$$f = \frac{100 \log_e 2}{t_{\frac{1}{2}}} = \frac{69 \cdot 3}{t_{\frac{1}{2}}}$$

The 30 keV and 80 keV curves were compared using the spectrum subtraction technique (Crawley and Veall, 1971) to give the activity/time curve from a smaller volume of tissue within the placenta. This technique was particularly sensitive to shift caused by fetal movements or changes in uterine muscle tone so the resulting curves were subject to wide fluctuations, did not improve the accuracy of measurement and were therefore discarded.

Maternal blood pressure was recorded by auscultation over the brachial artery with a standard sphygmomanometer cuff around the right upper arm. Recordings were taken before and at five minute intervals during each placental blood flow measurement. The average systolic and diastolic pressure was taken to represent the blood pressure (BP) relating to

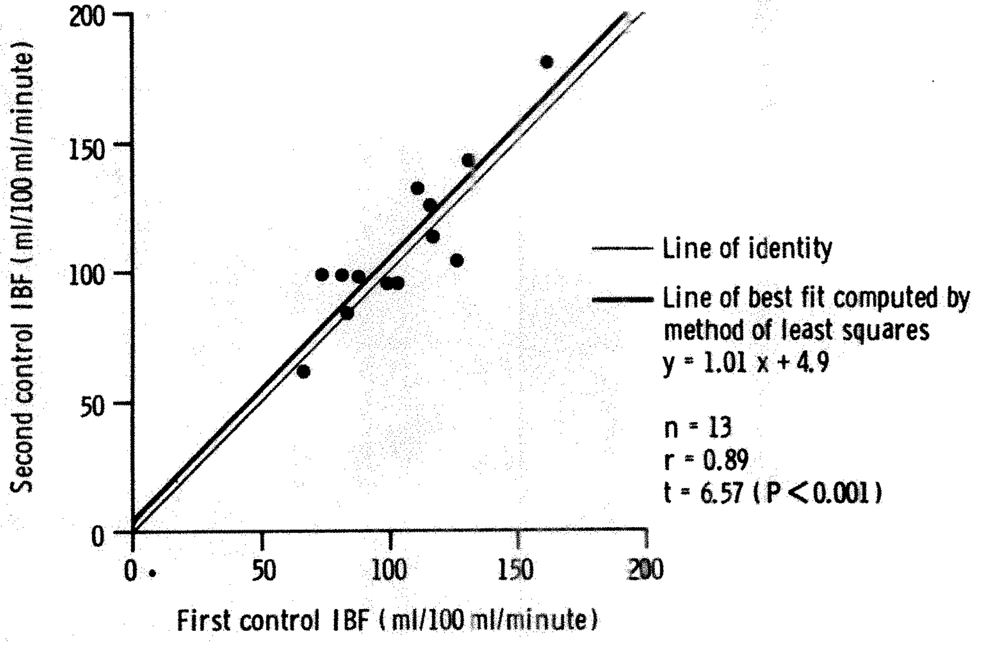

FIG. 1

Correlation between first and second control intervillous blood flow (IBF) measurements using ^{133}Xe inhalation method.

each of the respective placental blood flow measurements. The BP was then expressed as mean arterial pressure (MAP) according to the formula $\text{MAP} = \text{Diastolic pressure} + \frac{\text{Pulse pressure}}{3}$ (Ganong, 1973).

Statistical analysis of the recorded data is by the paired and unpaired t test as appropriate.

RESULTS

The reproducibility of IBF measurement with this ^{133}Xe inhalation method is indicated in Figure 1. The mean difference between first and second control IBF values was 11·2 per cent.

Data relating to the 18 recorded measurements of IBF before and after induction of epidural analgesia with 37·5 mg bupivacaine are set out in Table I. Where two control measurements of IBF were made before epidural analgesia the second control values for BP, MAP and IBF were used for comparison with post-epidural values.

The overall mean IBF before epidural block was 98·3 ml/100 ml/minute and after 94·9 ml/100 ml/minute. There was no statistically significant difference between paired IBF values before and after epidural block ($P > 0{\cdot}1$). Although there was a statistically highly significant difference between paired MAP values before and after epidural block ($P < 0{\cdot}01$) the mean MAP after epidural block (80 mm Hg) was only 6 mm Hg lower than mean MAP before (86 mm Hg). In no instance did the systolic blood pressure fall more than 30 mm Hg at any time, nor did any patient develop any symptom or sign of hypotension after epidural block. There was no significant correlation between change in MAP and percentage change

TABLE I

Clinical information about patients and details of intervillous blood flow (IBF) measurements before and after epidural block

				Before epidural analgesia (control)			After epidural analgesia		
Maternal age (years)	Indication for induction of labour	Weight of infant (g)	Weight of placenta (g)	BP (mm Hg)	MAP (mm Hg)	IBF (ml/100 ml/minute)	BP (mm Hg)	MAP (mm Hg)	IBF (ml/100 ml/minute)
30	Static weight	3280	675	90/60	70	51	80/50	60	53
28	Previous large baby	3260	600	105/65	78	116	105/65	78	105
38	Maternal age; large fetus	3780	700	130/80	97	62	115/65	82	61
28	Breech presentation	3020	650	95/70	78	114	80/50	60	89
30	Breech presentation	3380	500	115/75	88	133	115/70	85	123
32	Prolonged pregnancy	4150	750	110/65	80	96	110/65	80	104
28	Hypertension	4100	750	100/60	73	105	100/70	80	91
28	Hypertension	3020	550	125/75	92	143	120/60	80	119
25	Hypertension	3610	800	115/80	92	121	105/75	85	92
27	Hypertension	3450	650	130/70	90	83	120/60	80	115
25	Hypertension	3280	600	120/90	100	99	95/75	82	92
24	Hypertension	3060	550	135/75	95	84	140/75	97	102
26	Hypertension	3400	600	140/85	103	182	150/90	110	168
21	Suspected fetal growth retardation	*2640	600	100/70	80	60	95/65	75	73
32	Suspected fetal growth retardation	*2600	525	110/70	83	96	105/60	75	90
32	Twin pregnancy (monochorionic)	2640 2160	900	120/70	87	57	115/65	82	64
29	Twin pregnancy (dichorionic)	2500 2760	500 600	105/60	75	68	105/60	75	94
23	Twin pregnancy (dichorionic)	3120 3360	600 600	120/90	100	99	100/70	80	73

* Female infants, gestational age 40 weeks.
MAP, mean arterial pressure.

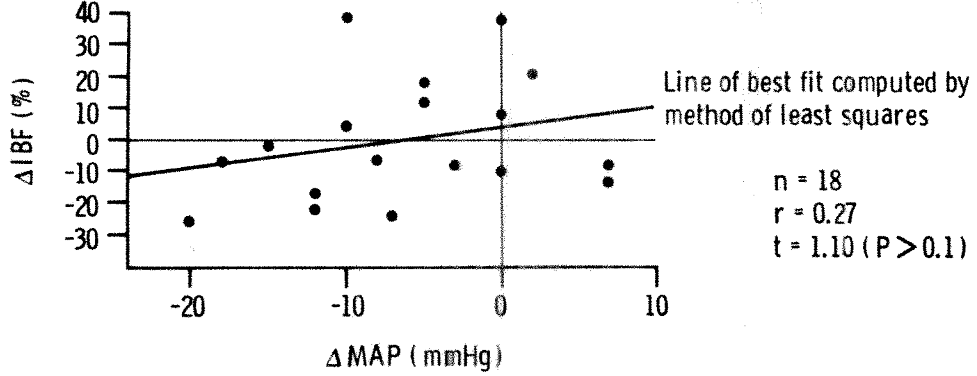

FIG. 2

Change in intervillous blood flow (IBF) (per cent) related to change in mean arterial pressure (MAP) (mm Hg) following epidural block.

in IBF before and after epidural block although the largest fall in MAP (20 mm Hg) was accompanied by the largest percentage fall in IBF (26 per cent) (Fig. 2).

There was no significant correlation between placental weight and IBF values.

For seven of the patients studied, the indication for induction of labour was hypertension during pregnancy but only two of these patients had a diastolic blood pressure greater than 80 mm Hg at the time of induction. The IBF values of the seven hypertensive patients before and after epidural block were not statistically different from corresponding IBF values of six normotensive women with normal birth weight singleton fetuses.

The two singletons with birth weight below the 10th centile for gestational age (Gairdner and Pearson, 1971) had control IBF values within the range of control values for normotensive singleton pregnancies of normal birth weight. In one instance the IBF increased by 17·8 per cent and in the other IBF decreased by 6·3 per cent following epidural block.

Three twin pregnancies were studied, one mono- and two di-chorionic. Control IBF values in the twin pregnancies were within the range of control IBF values for normotensive singleton pregnancies of normal birth weight. Following epidural block the percentage change in IBF for the two dichorionic twin pregnancies (−26·3 per cent and +38·3 per cent) were outside the range of change for corresponding values in normotensive singleton pregnancies of normal birth weight (−21·9 to +9·3 per cent).

DISCUSSION

The first attempts to obtain direct quantitative measures of placental IBF involved the injection of diffusable tracers into the intervillous space (Browne and Veall, 1953; Dixon *et al*, 1963; Clavero *et al*, 1973). Jacoby *et al* (1972) used ^{133}Xe, inhaled at a concentration of 0·5 mCi per litre for two minutes and Rekonen *et al* (1976) described a method in which they injected 2 mCi ^{133}Xe in isotonic saline intravenously after which the subjects held their breath for 20 seconds. In both inhalation and intravenous ^{133}Xe techniques, the isotope is delivered over an extended period of time rather than as a bolus so that the results are affected by recirculation. In order to make a correction for recirculation in cerebral blood flow measurements, Veall and Mallett (1966) measured the concentration of ^{133}Xe in expired air and their technique has subsequently been improved (Obrist *et al*, 1967; Abrams *et al*, 1969; Crawley and Veall, 1975). The agreement reported here between first and second control values, correcting for recirculation compares favourably with results reported by Rekonen *et al* (1976) and Joupilla *et al* (1978*a*). The estimated radiation dose was approximately 10 millirads to the maternal lungs and less than 1 millirad to the fetus.

A disadvantage of previously described methods and of the method described here is that blood flow is measured within a relatively small area of the placenta, expressed as flow per unit volume of placenta rather than total IBF. Even slight axial rotation of the uterus will alter the area of placenta being measured. If IBF is not uniform throughout the placenta this will introduce errors, particularly between repeated measurements but also within individual measurements. Kuikka *et al* (1978) found that IBF in the central part of the placenta was on average 55 per cent higher than in marginal parts.

Previous investigators of IBF in relation to epidural analgesia have used either relatively large doses of lignocaine for Caesarean section (Joupilla *et al*, 1978*a*), or relatively small doses of bupivacaine in patients receiving oxytocin infusions (Joupilla *et al*, 1978*b*). We report a study of IBF in relation to a larger dose of bupivacaine, which is widely used, in patients prior to induction of labour for various obstetric complications. In the relatively small number of patients investigated, induction of epidural analgesia with 10 ml of 0·375 per cent bupivacaine (37·5 mg) without adrenalin did not produce a statistically significant change in IBF compared with control values before epidural block under the conditions described. It cannot, however, be assumed that repeated 'top-ups' of epidural analgesia would not produce cumulative changes in IBF which might reach significant proportions; nor can it be assumed that an initial dose of 37·5 mg bupivacaine would not produce a significant change in IBF under circumstances different to those described here. There was no statistically significant correlation between changes in maternal blood pressure and IBF following epidural block, but clinically significant hypotension did not occur in any of our patients. We can not assume therefore that IBF is unrelated to maternal blood pressure over a wider range. In this respect it would also be interesting to determine whether a significant change in IBF occurs when epidural block produces a reduction of blood pressure from abnormally high levels to within normal limits in patients with severe hypertension.

ACKNOWLEDGEMENTS

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FETAL BREATHING PATTERNS IN INTRAUTERINE GROWTH RETARDATION

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Summary

Fetal breathing movements were studied with real time ultrasound in 21 normal fetuses and 30 fetuses born small-for-dates (less than the 5th centile). The events of the study period were recorded on punched paper tape which was subjected to computer analysis. In the normal group the percentage time of breathing was 54±12·7 (mean±SD). The breath to breath interval was 1·34±0·25 seconds and the coefficient of variation of this interval was 49·6±7·7 per cent. In 17 of the small-for-dates group amount of breathing was reduced with short breathing episodes and long apnoea intervals. In the other 13 patients in the small-for-dates group the coefficient of variation for the breath to breath interval was significantly reduced (39·6±7·9 per cent, $p < 0·01$).

BODDY and Robinson (1971) stimulated interest in fetal breathing movements as a possible indicator of fetal wellbeing. Some studies have shown that diminution or absence of breathing movements may predict fetal compromise or death (Boddy and Dawes, 1975; Manning, 1977). In a similar study using real time ultrasound we found that diminished breathing movement was most commonly associated with poor fetal outcome, but in some fetuses the amount of breathing was in the upper normal range (Trudinger *et al*, 1978). It was suggested that observation of the pattern of fetal breathing may be of more diagnostic value than measuring the amount of time fetuses spend breathing. In sheep, three abnormal patterns of breathing have been described: apnoea with gasping, brief episodes of breathing with gasping and a pattern of continuous very regular breathing (Patrick *et al*, 1976). The present study examined both the amount and regularity of fetal breathing in fetuses with intrauterine growth retardation.

METHODS

Patients

Fetal breathing was studied in 21 normal pregnant women and in 30 patients with fetal growth retardation. All patients were studied after the 33rd week of gestation. In the normal group, maternal blood pressure never exceeded 140/90 mm Hg, the pregnancy was uncomplicated and fetal growth as measured by ultrasound in the last four weeks of pregnancy was normal. There was no fetal distress in labour and all patients were delivered of a single infant of a birth weight greater than the tenth centile. One and five minute Apgar scores were 8 or greater.

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The 30 patients with fetal growth retardation were selected from a larger population chosen because they were undergoing serial ultrasound studies for suspected intrauterine growth retardation. Each of the 30 patients finally selected was delivered of a child whose birth weight was below the fifth centile for the mother's height, weight, parity and sex of child (Tanner and Thomson, 1970). The mean study to delivery interval was 6·5 days (range 1 to 21 days).

Fetal breathing measurements

A sagittal section of the fetal chest was scanned with a real time scanner (ADR 2130 Kretztechnik) using a 3·5 mHz transducer. Fetal breathing movements were recognised as an inward movement of the anterior chest wall accompanied by a downward descent of the diaphragm and outward movement of the anterior abdominal wall and return. Each study period lasted 30 minutes and a video recording of the real time image was made. Each patient was studied 30 to 45 minutes after a 50 g oral glucose drink (Lewis *et al*, 1978). Abdominal palpation before the study was avoided and no patient was studied after amniocentesis. Patients were asked to refrain from smoking on the study day. The video tape was subsequently analysed by a single trained observer, who registered each fetal breath and period of whole body movement by pressing the appropriate button of a hand held tachometer. This was connected to a data logger which recorded both the nature of the event and the lapse of time from the start of the recording. The different variables of fetal breathing activity were obtained by a computer analysis of the punched paper tape. The fetal breathing index (FBI) was the percentage of time continuous breathing occurred (interval between successive breaths less than 6 seconds) corrected for the time the fetus was moving or otherwise unsighted. The mean breath-to-breath interval and its standard deviation were computed for intervals of less than 6 seconds. The coefficient of variation of the breath-to-breath interval was used as a measure of variability thus correcting for the tendency for the standard deviation to decrease with the mean.

TABLE I

Reproducibility of repeated analysis of the same video-recordings. The coefficient of variation is the mean figure derived from 3 studies; in each study there were five repeat analyses of a 30-minute reading

Variable	Coefficient of variation (per cent)
Fetal breathing index	8·2
Breath-to-breath interval—	
mean	3·5
standard deviation	5·8

Reproducibility of the method was tested by analysis of the same videorecording on five separate occasions at least one week apart. This test was repeated three times with the observer blind to the testing during two studies. These results are shown in Table I.

RESULTS

The fetal breathing index (FBI) for the 21 normal fetuses was 54±12·7 (mean±SD). For this population the mean breath-to-breath interval was calculated to express rate whilst the mean standard deviation and coefficient of variation (SD/Mean) provided a measure of regularity of rate. The breath-to-breath interval was 1·34±0·25 seconds (mean±SD) and the standard deviation for the breath-to-breath interval was 0·66±0·16 seconds (mean±SD). The variability of breathing rate was high, the coefficient of variation being 49·6±7·7 per cent. The 5th and 95th centile values for the FBI for the normal fetuses are shown in Figure 1.

The distribution of FBIs for the small-for-dates group was bimodal (Fig. 1). A reduced amount of breathing was the more common pattern but there was a group of abnormal fetuses whose FBI was higher than the 95th centile of the normal population. The important additional diagnoses for the compromised fetuses are indicated on Figure 1. There were two perinatal deaths; both occurred in the early neonatal period and were directly attributable to problems of severe growth retardation. Only four fetuses developed fetal distress in labour (abnormal fetal heart rate trace or acidotic scalp

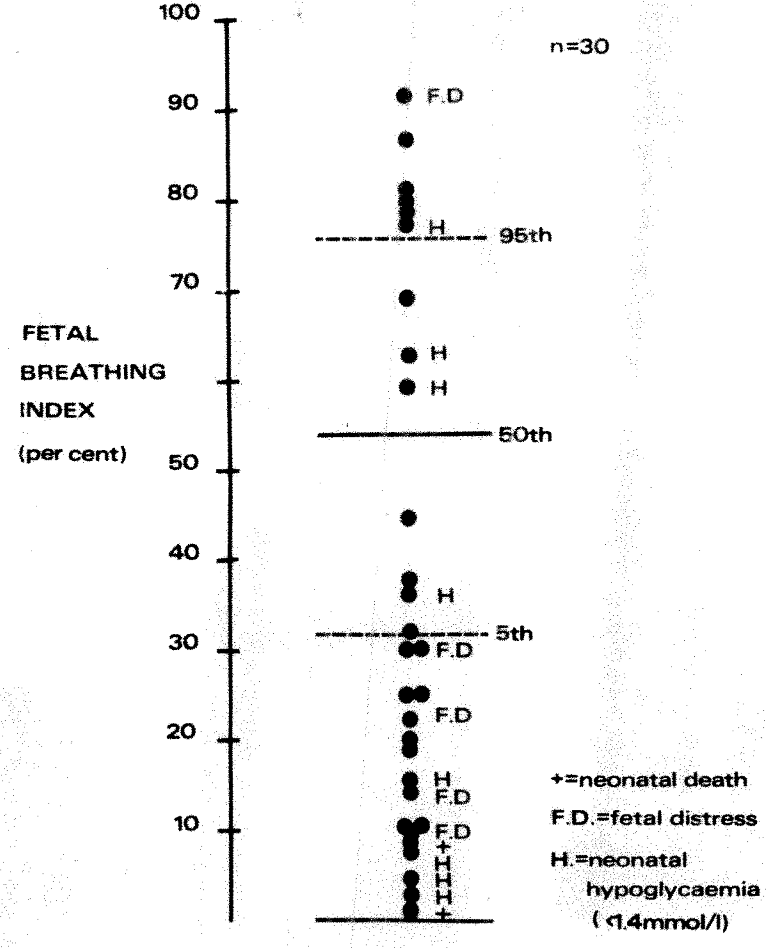

FIG. 1

Distribution of fetal breathing index (FBI) in the 30 small-for-dates fetuses (less than 5th centile). The 5th, 50th and 95th centile lines for the FBI are derived from the 21 normal fetuses studied.

blood sample necessitating delivery). Eight infants developed hypoglycaemia, (less than 1·4 mmol/l) in the early neonatal period. Seven patients were delivered electively by Caesarean section for suspected placental insufficiency; the two neonatal deaths occurred in this group and four of the babies developed neonatal hypoglycaemia.

In the normal fetuses, the mean number of breaths in each breathing episode was 25 and the mean episode time 30 seconds. In the abnormal group, those infants having an FBI below the 5th centile had a mean of 10 breaths per episode and a mean episode time of 13 seconds. These episodes were too short to derive data on breath-to-breath intervals. The eight growth retarded fetuses with an FBI value falling within the normal range showed no significant difference from the control group (mean breaths per episode 30, episode time 37 seconds) while the five abnormal fetuses whose FBI was above the 95th centile had a greater mean number of breaths in each episode (98) and a longer mean episode time (120 seconds).

The mean breath-to-breath interval of the growth retarded group was not significantly different from the control group. However, the coefficient of variation of the breath-to-breath

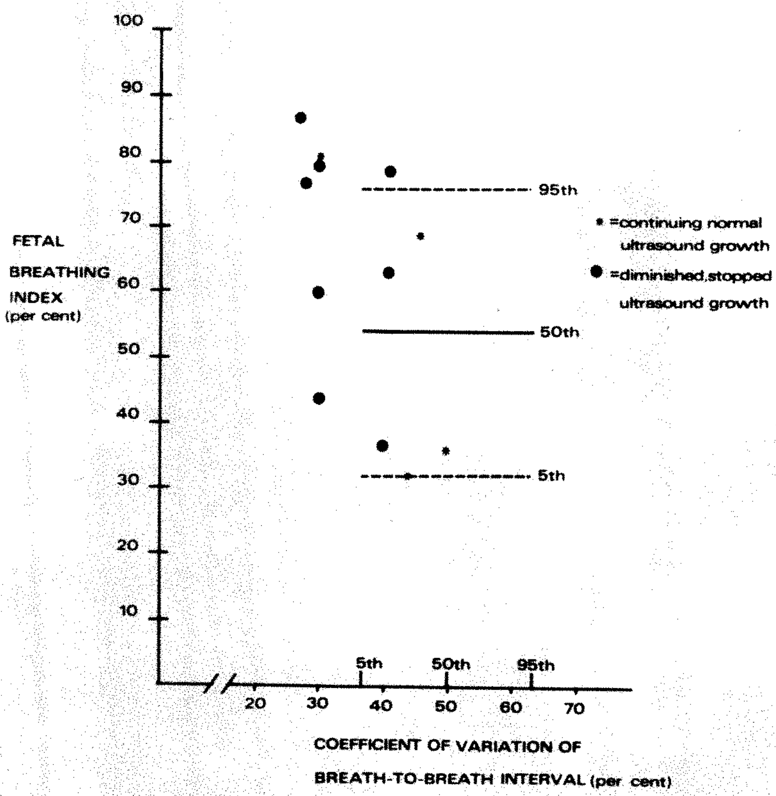

FIG. 2

Distribution of the coefficient of variation of the breath-to-breath interval in 12 of the 13 small-for-dates fetuses with an amount of breathing greater than the 5th centile. In one case there was insufficient data for ultrasound analysis. The centile lines are derived from the control group.

interval of those in this group with an FBI above the 5th centile value was $39 \cdot 6 \pm 7 \cdot 9$ per cent a value significantly less than the control group ($p < 0 \cdot 01$). This result is shown in Figure 2 and indicates a more regular rhythm of breathing amongst those small-for-dates fetuses in whom the actual amount of breathing was not markedly reduced.

DISCUSSION

Fetuses born small-for-dates presumably have a poor intrauterine environment. It might thus be expected that these fetuses would show differences in breathing movements. We have earlier reported (Trudinger *et al*, 1978) that growth retarded fetuses tend to show either diminished or excessive fetal breathing. This report extends these observations and attempts to look more closely at the variables of frequency and regularity of breathing. In the compromised group, those with a reduced amount of breathing had episodes of breathing which were shorter than normal. The occurrence of such short episodes of breathing prevents comment on regularity of breath-to-breath intervals with our method. The acceptance of a breath-to-breath interval of up to 6 seconds as continuous breathing enhances variability when the number of breaths is low. In these growth retarded fetuses showing the other pattern, excessive breathing, the pattern of breathing was abnormally regular, showing reduced variability of breath-to-breath intervals compared with normal fetuses.

These observations have not been reported previously as most workers have concentrated on the percentage of time that fetal breathing was present (Bots *et al*, 1978). However, the abnormally regular pattern of fetal breathing in those fetuses in whom breathing was not reduced is of particular interest since it resembles observations made in studies of sheep. In the chronic fetal lamb preparation, a monotonous breathing pattern referred to as 'picket fence' breathing because of the regularity of the record is seen sometimes as a terminal pattern (Patrick *et al*, 1976). More recently, maternal hypercapnoea without hypoxia has been reported to produce a similar regularization of fetal breathing in man (J. Ritchie and K. Lakhani, 1978, personal communication), a finding which parallels observations in the fetal lamb (Chapman *et al*, 1978).

The present studies support the view that measurement of other variables of human fetal breathing apart from the percentage of time the fetus spends breathing may provide some additional indications of fetal condition.

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BIRTH WEIGHT: ANALYSIS OF VARIANCE AND THE LINEAR ADDITIVE MODEL

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Summary

The normal distribution of birth weight in a Queensland population has been surveyed. A method of analysis is presented which allows for the influence of a number of factors that affect birth weight.

WE have attempted to find a standard table of birth weight appropriate to our local population, but in reviewing the literature were confronted with two problems. First, there is no large survey of birth weight in Australia, with the exception of Kitchen (1968) who surveyed 2009 births. All other series were of other nationals (McKeown and Gibson, 1951; McKeown and Record, 1953; Lubchenco *et al*, 1963; Gruenwald, 1966; Thomson *et al*, 1968; Cheng *et al*, 1972; Milner and Richards, 1974) and may be inappropriate for our population. Second, there are many factors other than gestation which affect birth weight, and some of the references quoted dealt with one or more of these factors. The use of multiple tables similar to those of Thomson *et al* (1968) are too cumbersome when more than one or two factors are taken into consideration.

With these problems in mind, we surveyed our own population to establish the normal birth weight distribution, using the multiway analysis of variance (Steel and Torrie, 1960) and the associated linear additive model to take account of the many factors which affect birth weight. The results of such a survey and analysis are presented in this paper.

METHOD

The computerized case records of all patients who were delivered at the Mater Mothers' Hospital, South Brisbane, between April 1972 and June 1976 were reviewed, and all patients with a recorded infant birth weight were selected for analysis. Babies delivered before 28 weeks and after 42 weeks were excluded, as the numbers in each gestational group were too small for proper analysis. Patients with multiple pregnancy, fetal abnormality, and macerated stillbirths, were also excluded as the babies were unrepresentative of the overall population.

A number of factors were identified, and their effects on birth weight analysed. Gestation was calculated on the basis of the last menstrual period. Where there was uncertainty, the best clinical estimate prior to delivery was used. The mothers' countries of origin were initially divided into many groups, but finally allocated to three groups: Australian whites and all other Europeans other than the Mediterranean countries were in one group; Italians, Greeks, Maltese, and Spanish were in the second; and Aboriginal and Pacific Islanders in a third. The number of Asians and miscellaneous nationals were so few that they were not analysed separately, but included in the predominant Australian white group. The age, height, weight, and parity were recorded; the height-adjusted weight was divided into three groups as thin (less than 0·357 kg per cm), average, and obese (more than 0·519 kg/cm). These represented the 10th and 90th centile of height-adjusted weight in our population. The weights used were the maximum weight recorded during the pregnancy.

The classification according to socio-economic background was difficult as relationship between income and work status are different to the standard British classifications. The patients were divided into public and private patients (58·1 per cent: 41·9 per cent), as this classification roughly corresponds to socio-economic status.

An initial analysis, calculating the mean, standard deviation, and centiles of weight for each gestation was made. A further analysis, using patients who were delivered between 36 and 42 weeks gestation was made using the multiway analysis of variance, so that the overall average, the variance attributed to different factors, and the loading attributed to them were calculated. Interactions were calculated only between gestation and various factors, but they were not statistically significant, and were left out of the presentation. Multiple interaction of all factors was not made, as the number of subgroups would have been too large, and the numbers in each subgroup too small.

A total of 4784 patients (34·1 per cent) was excluded in this second analysis because one or more of the relevant factors was not recorded. At the end of all calculations, each individual birth weight was compared with its theoretical weight by z conversion to standard deviation units and the distribution re-examined. The model was validated as 9·9 per cent had weights less than −1·28 standard deviations from the mean (10th centile), 10·3 per cent had weights greater than 1·28 standard deviation from the mean (90th centile), and 79·8 per cent in between. A Chi square test showed that this did not deviate significantly from that expected in a normal distribution.

RESULTS

A total of 14 328 birth weights was extracted for initial analysis, and the results are presented in Table I and Figure 1. The weights at 29 and 31 weeks were included in their preceding weeks in Figure 1 because of small numbers.

TABLE I

Table of standard birth weight (g) taking only gestation into consideration

		Gestational age (weeks)															
		28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	36–42*
Number		19	6	21	5	41	27	70	93	280	511	1343	2379	6037	2338	1158	9262
Mean		1240	1564	1471	1500	1743	1992	2205	2357	2664	2915	3172	3337	3474	3554	3565	3440
Standard deviation		540	381	405	275	392	385	366	462	504	451	470	449	455	458	485	425
Centile	10	549	1076	952	1147	1254	1500	1737	1765	2020	2337	2571	2762	2892	2968	2945	2896
	20	802	1255	1143	1277	1433	1681	1909	1982	2256	2549	2792	2973	3106	3183	3173	3096
	30	959	1366	1260	1356	1544	1792	2015	2116	2402	2680	2928	3103	3237	3316	3313	3219
	40	1105	1469	1370	1431	1647	1896	2114	2241	2538	2802	3055	3225	3360	3440	3444	3334
	50	1240	1564	1471	1500	1743	1992	2205	2357	2664	2915	3172	3337	3474	3554	3565	3440
	60	1374	1659	1573	1568	1838	2088	2297	2472	2790	3028	3290	3449	3587	3669	3687	3546
	70	1520	1762	1682	1643	1941	2192	2396	2597	2926	3149	3417	3570	3710	3792	3818	3661
	80	1677	1873	1800	1723	2052	2304	2502	2731	3072	3280	3553	3700	3842	3925	3958	3784
	90	1930	2052	1990	1852	2232	2485	2674	2948	3309	3493	3774	3912	4056	4140	4186	3984

* Patients at 36 to 42 weeks for whom all relevant data for analysis in Table II were available.

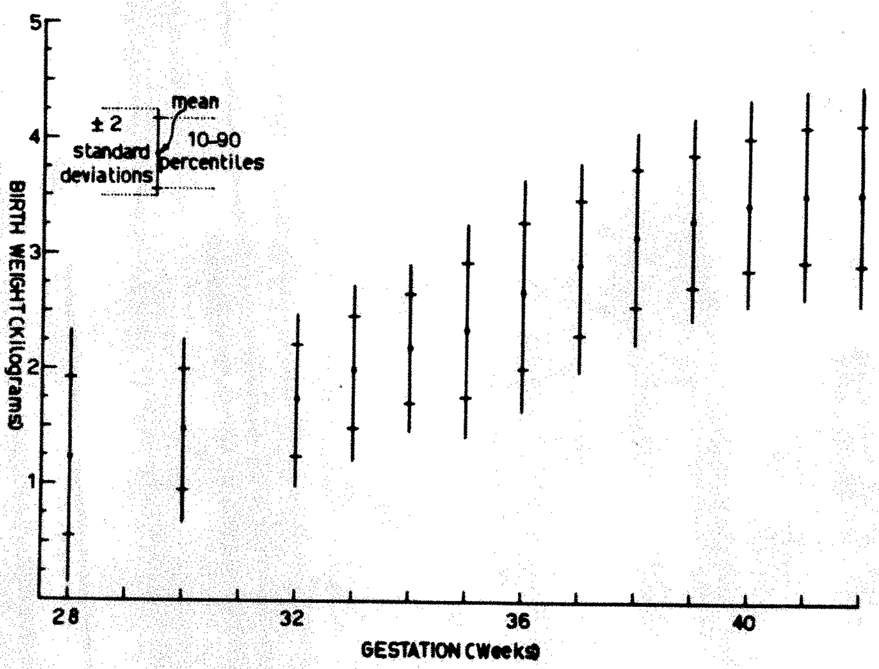

FIG. 1

Birth weight distribution in different gestational ages.

TABLE II

Multiway analysis of variance without interaction (unit = kg)

	Degrees of freedom	Sum of squares	Mean square	F
Gestation	6	434·60	72·43	402·39
Age	5	84·14	16·83	93·50
Public or private patient	1	20·63	20·63	114·61
Height	5	61·60	12·32	68·44
Height adjusted weight	2	42·50	21·25	118·06
Country of origin	2	1·96	0·98	5·44
Parity	5	31·32	6·26	34·78
Sex	1	78·16	78·16	434·22
Residual	9234	1647·44	0·18	
Total	9261	2402·35		

A total of 9262 birth weights of babies delivered from 36 to 42 weeks was used for the analysis of variance. The mean and standard deviation are presented in Table I, the analysis of variance is presented in Table II, and the loading weights in Table III.

Gestation was the most important factor affecting birth weight, as would be expected from Table I. The range was 901 g (−742 to +159).

Maternal height was the second most important factor, increasing height being related to increasing birth weight, with a range of 364 g (−238 to +126).

Height adjusted weight was used in preference to weight alone, to minimize the effect that height had on weight. The three categories could reasonably be summarized as thin, average, and obese. Birth weight increased with increasing weight of the mother. The range was 341 g (−288 to +53).

Parity. Birth weight was directly proportioned to parity, being lowest in the primigravidae, highest in the grand multiparae. The range was 154 g (−56 to +98).

Sex. Male babies weighed more than female babies, with a range of 150 g (+74 and −76 respectively).

Country of origin had a range of 125 g. Whites from Australia, New Zealand, United Kingdom

TABLE III

Table of loading factors for linear equation

Fetal weight 36 to 42 weeks	Calculated from 9262 patients (see last column of Table I)
Mean = 3440	SD = 425 (residual)
10th centile = 2896	90th centile = 3984

Formula:
Mean fetal weight = 3440 + Σ (loading weight) g

Loading factor		Loading weight (g)
Gestation (weeks)	36	−742
	37	−491
	38	−234
	39	− 69
	40	68
	41	148
	42	159
Maternal age	≤19	− 63
	20–24	− 30
	25–29	30
	30–34	56
	35–40	50
	>40	− 48
Patient status	Public	− 26
	Private	37
Height (cm)	≤150	−238
	150–155	−114
	155–160	− 33
	160–165	12
	165–170	43
	>170	126
Height adjusted weight	Thin	−288
	Average	− 50
	Obese	53
Country of origin	Australian white, U.K., Northern European	5
	Australian Aborigines	−120
	Southern European	− 28
Parity	0	− 56
	1	19
	2	48
	3	54
	4	65
	5+	98
Sex of infant	Male	74
	Female	− 76

and Northern Europe (+5 g) hardly differed from Southern European Whites (−28 g). Although the survey did not include a large number of Australian Aborigines (102), their birth weight was slightly lower (−120 g).

Maternal age. Birth weight increases with maternal age up to the 30 to 34 year age group, and then began to decline slightly. The range was 119 g (−63 to +56).

Patient status. A difference of 63 g (−26 and +37) existed between the private and public patients. This represents a small but significant difference.

DISCUSSION

Two areas of contention existed while the data was being sorted: what constitutes an abnormality sufficient to be misleading if it is included, and should patients with 'unsure' dates be excluded?

In this survey we have excluded multiple pregnancies, macerated stillbirths and gross fetal abnormalities on the grounds that they are so unrepresentative as to detract from the accuracy of assessing birth weight. We also excluded pregnancies of less than 28 and more than 42 weeks because of insufficient numbers. We included all other patients, whether with normal or pathological pregnancies, on the basis that we were trying to establish a normal range of birth weight for the local population, and not for a population with entirely normal pregnancies. The effect of various diseases and complications of pregnancy, including cigarette smoking, on birth weight, will be the subject of a further survey.

The question of including women with unsure dates is a vexed one. Some surveys include only those pregnancies in which gestation is certain (Thomson *et al*, 1968; Cheng *et al*, 1972); some include some categories of unsure dates, but exclude others (Lubchenco *et al*, 1963; Gruenwald, 1966); others include both certain and uncertain dates (Kitchen, 1968; Milner and Richards, 1974). We have included all patients, using menstrual data, or failing that the best clinical estimate before delivery. Since the advent of oral contraception, the number of patients with unsure menstrual data has increased considerably. To exclude such a large group would run the risk of serious bias in the population being surveyed.

Only birth weights from 36 to 42 weeks were included in the more detailed analysis because the numbers in the other gestation groups were insufficient for the analysis of multiple factors.

We investigated those factors which have previously been shown to affect birth weight. Barron and Vessey (1966) reviewed the literature and found a correlation between birth weight and gestation, sex of infant, parity, race, height and weight. Age was considered a 'doubtful' factor, and maternal socio-economic status complex and probably related to stature.

The importance of considering all of these factors in conjunction is graphically illustrated by considering the overall mean birth weight at 40 weeks: 3474 g (from Table I). However, when one introduces the other factors (Table II), a young, thin, short Aboriginal primigravid public patient will be expected to produce a female baby at 40 weeks with a mean birth weight of 2641 g (3440 + 68—63—26—238—288—120—56—76) whilst an older, fat, tall, white Australian grand multiparous private patient would be expected to have a mean birth weight for a male baby of 3957 g at 40 weeks (3440+68+56+37+126+53+5+98+74).

When considering birth weight, the clinician is usually more interested in whether a particular baby is below the 10th or above the 90th centile. A similar calculation can be performed, using the linear additive model, but with the fetal weight appropriate to the desired centile from Table I. In the examples already cited, the 10th centile for the Aboriginal baby is 2097 g (2896+68—63—26—238—288—120—56—76), and for the white baby 3413 g (2896+68+56+37+126+53+5+98+74). The corrected 90th centile for each would be 3185 g and 4501 g.

These two extremes, with a difference of means of 1316 g, show the error of considering birth weight only in relation to gestation. From Table I, using 3474 g as the mean, the first patient could be below the 10th centile and the second patient between the 80th and 90th centile.

Some authors take into account one or more of the factors which we have considered when constructing tables (McKeown and Record, 1953; Lubchenco *et al*, 1963; Thomson *et al*, 1968; Cheng *et al*, 1972; Milner and Richards, 1974), whilst others do not (Gruenwald, 1966; Kitchen, 1968). Thomson *et al* (1968) considered the most factors: height, weight for height, parity, social class, and sex of baby, besides gestation, and produced a complex series of tables. From these it is possible to calculate a mean birth weight taking into account three variables in conjunction. But despite the number of tables, it is not possible to view birth weight in more than a three-dimensional view. Using the linear additive model, any number of factors can be considered, and whenever a factor is not obtained, the allocation of that particular variance to the residual and the recalculation of residual standard deviation will readjust the correct weight with available information.

In our calculation, we presumed that the patients were randomly distributed in all factors, and that the factors operated independently, without interaction. These presumptions might have been wrong, especially when considering weight, parity, and age, when they might have been related. However, to break the classes down would require 54432 sub-groups—an impracticable undertaking with the numbers available in hospitals. This simpler model represents a compromise between statistical precision and practical requirements.

Finally, our data pertains only to Brisbane women, and may be of limited value to workers in other populations. However, our method of formulating mean birth weights is readily adaptable for use in other centres, and with any number of different variables.

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BIRTH WEIGHT OF HAUSA INFANTS IN NORTHERN NIGERIA

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Summary

The birth weight of 1460 Hausa singleton infants was recorded and analysed. The birth weight of Hausa infants (Mean ± SD) was 3·03 ± 0·45 kg, being higher in males (3·08 ± 0·47 kg) than females (2·97 ± 0·48 kg). The difference was significant ($P < 0{\cdot}001$). The mean birth weight of first born children was significantly lower than the subsequent born, and increasing birth order was assoicated with higher birth weight irrespective of maternal age. The birth weight was influenced more by the parity as compared to maternal age. The incidence of low birth weight was 15·8 per cent, being lower in males (13·6 per cent) as compared to females (18·1 per cent). Comparison were made with studies from other parts of the country as well as from other countries.

ALTHOUGH birth weight is a well recognised index of intrauterine growth (Lubchenco, 1970) few data are available from developing countries, where the widely used standards from Western countries are inappropriate. In a country like Nigeria, which has well defined ethnic diversities, there is an obvious need for local standards. Some data have been collected for the southern part of the country (Morley and Knox, 1960; Effiong *et al*, 1976; Oduntan and Ayeni, 1976; Ladipo and Adelusi, 1977) but information from the northern regions, particularly about the Hausa children, is almost non-existent except for one report, which dealt with a mixed population (Osuthor, 1976). There are considerable ethnic, religious, socio-economic and cultural differences between the southern and northern parts of the country which are known to influence all aspects of life including birth weight (Harper, 1976).

The present study was undertaken to provide information about the mean birth weight of Hausa children in this northern town, and to determine the incidence of low birth weight and its relation to maternal age and partiy.

SOCIAL AND CULTURAL BACKGROUND

Hausa is a linguistic, not an ethnic term, and refers to those whose mother tongue is the Hausa language (Hill, 1972). The Hausas come from varied ancestries and adoption of a common language has been the essential factor in binding them together as one people. In the northern savanas, intermarriage between Negroes and Hamitic people—a branch of the Mediterranean race, named after Ham, the second son of Noah—gave rise to the tribes of mixed blood. The most numerous of these people of mixed stock are the Hausas (Perkins and Stembridge, 1966).

The Hausa people are settled mainly between

10·5 and 13·5 degrees North and 4 to 10 degrees East, in a huge area in the north of Nigeria and the south of Niger Republic. The Hausaland consists essentially of the basin of Sokoto river and its tributaries to the west and of a greater plateau to the east (Hill, 1972). Good farmers and skilled craftmen, the Hausas are known throughout Africa as remarkably clever traders.

The height and weight (Mean ± SD) of the men aged 20 years and over, in this area, is176 ± 10 cm and 63 ± 10 kg (Collard, 1962). The corresponding figures recorded during our recent field surveys were 175 ± 9 cm and 58 ± 8 kg (Abdullahi and Rehan, unpublished). Collard (1962) reported the height and weight (Mean ± SD) of women of the same age as 155 ± 8 cm and 55 ± 6 kg while the values recorded by us were 157 ± 8 cm and 55 ± 6 kg.

In most parts of Hausaland, child marriage is the rule; both boys and girls are married at the age of 12 to 13 years in the large towns but at even younger ages in the villages (Madauci *et al*, 1968). Girls may be married before they reach puberty, so that some have their first menstrual period when they are already in their marital homes (Harrison, 1978). Consequently, most of the girls have their first child before they are fully grown. Being a predominantly Muslim society, polygamy is widely practised and only a minority of the women smoke, though the trends are changing with younger generations.

METHODS

The records of all deliveries conducted at Maternity Hospital, Katsina, between 1st January, 1975 and 31st December 1976, were studied and 1460 patients were selected for study because (i) the age of the mother was known; (ii) the date of last menstrual period (LMP) was precisely stated; (iii) the duration of gestation, calculated from the LMP was consistant with the clinical examination; (iv) the duration of gestation was 37 weeks or more at the time of delivery; (v) there was no pre-eclampsia, eclampsia or hypertension; (vi) there was no complication or surgical interference during labour; (vii) the birth weight of the singleton live infant was accurately recorded after delivery using a metric scale which was frequently checked for zero error.

All the women included in this study were Hausa, married to Hausa men. Although the classification of the Nigerian population into accurate socio-economic classes is difficult because of the peculiar social structure (Ladipo and Adelusi, 1977), we can say on the basis of the husband's occupation that all these women belonged to a low to middle socioeconomic background. Seventy-two per cent of the women had attended or were formally registered at the antenatal clinic; the other 28 per cent had not attended any antenatal clinic.

Table I gives the distribution of mothers

TABLE I

Distribution of mothers according to age and parity

	Maternal age (years)				
	18 or less	19–24	25–30	31–36	37 or more
Number (n = 1460)	350	521	470	89	30
Per cent	24	36	32	6	2

	Parity						
	0	1	2	3	4	5	6 or more
Number (n = 1460)	379	286	258	174	112	94	157
Per cent	26	20	17	12	8	6	11

according to age and parity. Age ranged from 14 to 41 years (Mean ± SD being 23·2 ± 6 years). Parity ranged from 0 to 8 with more primigravidae than any other parity.

The birth weights were calculated separately for the 758 male and 702 female children.

RESULTS

The birth weight (Mean ± SD) for these 1460 full term singleton children was 3·03 ± 0·45 kg, 3·08 ± 0·47 kg for males and 2·97 ± 0·48 kg for females. The difference was statistically significant (P <0·001).

Relation of the birth weight to the length of gestation

Table II shows the mean birth weight for each completed week of gestation: there was a significant increase in the birth weight up to 40 weeks, after which there was a slight tendency to decrease.

Relation of the age and parity of the mother to birth weight

The mean birth weight of the children born to the mothers of 18 years or less was significantly lower than the mean birth weight of the children born to older mothers (P <0·001). Although the figures in Table III indicate that mean birth weight is increasing with maternal age the differences at age groups beyond 24 years were not significant.

The analysis of mean birth weight according to parity is summarized in Table IV. The mean birth weight of firstborn children was significantly lower than those from second and

TABLE II

Mean birth weight by length of gestation

Completed weeks of gestation	Males		Females		Total	
	No.	Weight (kg) Mean ± SD	No.	Weight (kg) Mean ± SD	No.	Weight (kg) Mean ± SD
37	86	2·85 ± 0·50	78	2·75 ± 0·50	164	2·80 ± 0·50
38	163	2·96 ± 0·43	151	2·85 ± 0·41	314	2·90 ± 0·42
39	284	3·13 ± 0·45	265	2·99 ± 0·45	549	3·06 ± 0·45
40	162	3·20 ± 0·41	157	3·13 ± 0·48	319	3·16 ± 0·44
41 or more	63	3·15 ± 0·56	51	3·08 ± 0·53	114	3·11 ± 0·55

TABLE III

Mean birth weight by maternal age

Age (years)	Males		Females		Total	
	No.	Weight (kg) Mean ± SD	No.	Weight (kg) Mean ± SD	No.	Weight (kg) Mean ± SD
18 or less	187	2·90 ± 0·50	167	2·83 ± 0·42	350	2·87 ± 0·46
19–24	256	3·09 ± 0·41	265	2·98 ± 0·46	521	3·04 ± 0·44
25–30	252	3·15 ± 0·50	218	3·03 ± 0·65	470	3·10 ± 0·57
31–36	51	3·24 ± 0·60	38	3·12 ± 0·41	89	3·19 ± 0·53
37 or more	16	3·18 ± 0·68	14	2·83 ± 0·82	30	3·01 ± 0·69
Total	758	3·08 ± 0·47	702	2·97 ± 0·48	1460	3·03 ± 0·45

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subsequent pregnancies, with a progressive increase in the birth weight with rising parity, up to the fourth child.

Since the primiparae formed the largest single group and are of particular importance in view of the early ages of childbearing, it was thought worthwhile to study the mean birth weight of the firstborn children in more detail. These mothers were grouped according to their ages in two year intervals and minor statistical adjustments were made to ensure that the mean length of gestation and the sex ratio was about the same at each age group. The results, summarized in Table V, show that the smallest mean birth weight occurred in the youngest group: mothers of 14 and 15 years old, but the correlation of mean birth weight with age ($r = 0·52$) was not significant.

Since age and parity are closely related, further analysis was required to determine their relative effects. In Figure 1 the relation between age and birth weight is shown for six separate parity groups. While there is an overall tendency for lines of increasing parity to lie at higher levels, it is not possible to decide the relative contribution of the two variables to birth weight. The coefficient of correlation between mean birth weight and parity ($r = 0·96$) was highly significant ($P < 0·001$); that between mean birth weight and age ($r = 0·64$) was not significant suggesting that the change in birth weight with age and parity is predominantly a function of parity.

Incidence of low birth weight

The term 'low birth weight' was used for all those babies who weighed 2·50 kg or less at birth (WHO, 1961). Two hundred and thirty

TABLE IV

Mean birth weight by parity

	Males		Females		Total	
Parity	No.	Weight (kg) Mean ± SD	No.	Weight (kg) Mean ± SD	No.	Weight (kg) Mean ± SD
0	195	2·83±0·68	184	2·78±0·54	379	2·81±0·61
1	136	3·06±0·50	150	2·93±0·51	286	2·96±0·62
2	128	3·12±0·41	130	3·05±0·41	258	3·06±0·91
3	97	3·24±0·27	77	3·04±0·54	174	3·15±0·42
4	58	3·09±0·22	54	3·08±0·41	112	3·08±0·36
5	52	3·27±0·46	42	3·04±0·32	94	3·17±0·42
6 or more	92	3·24±0·45	65	3·27±0·39	157	3·25±0·43

TABLE V

Mean birth weight in first pregnancies by maternal age

	Maternal age (years)							
	14–15	16–17	18–19	20–21	22–23	24–25	26–27	28–30
Number	72	128	79	53	16	22	1	8
Weight (kg) Mean	2·74	2·82	2·90	2·84	2·84	2·96	3·22	2·81
SD	0·41	0·46	0·41	0·39	0·50	0·36	—	0·46

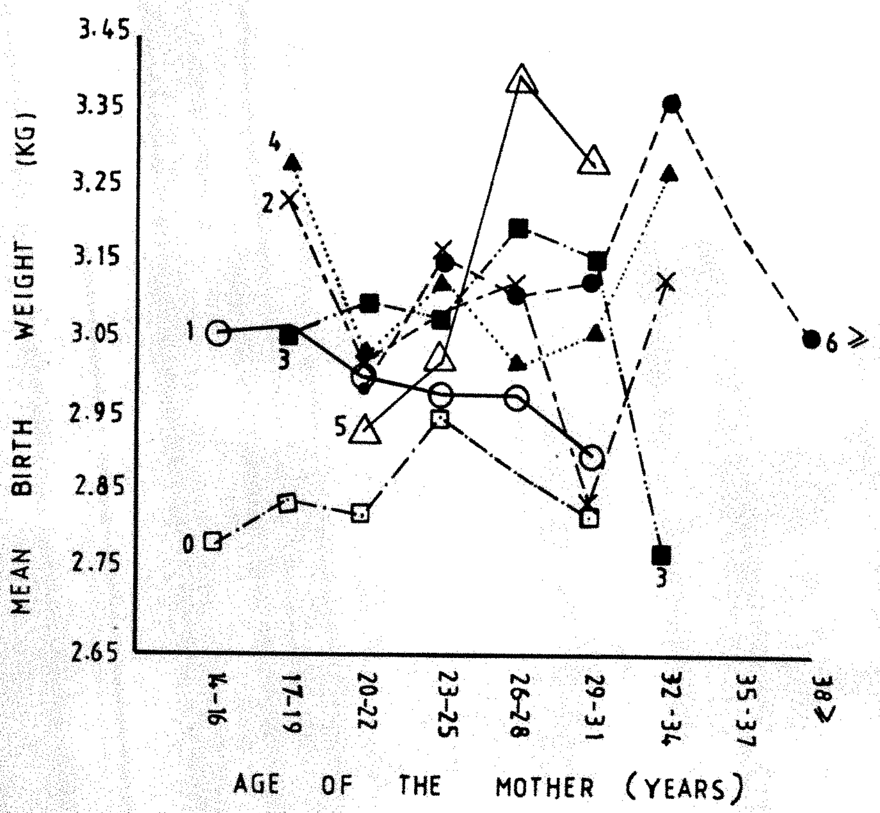

FIG. 1

Relation of mean birth weight to maternal age and parity (Parity is signified by numbers on the graph.

(15·8 per cent) of these full term infants weighed less than 2·50 kg. As expected, the incidence was higher in female children (18·1 per cent) than in male children (13·6 per cent); the difference was significant (P <0·001).

DISCUSSION

There is an inherent defect in relying on hospital data for biological standards in any community, but until compulsory registration and accurate vital statistics become available in developing countries, studies of this nature will continue to depend on hospital data (Effiong *et al*, 1976). Similarly our data might have an element of social selection, because of our insistance on knowing the LMP and the maternal age. However as we aimed at finding the mean birth weight of full-term children and its relation to variables which included maternal age, this element of 'bias' was unavoidable. As an example, our data might suggest that there are relatively few high parity Hausa women. This is not so, and the deficiency is due to our selection criteria.

Effiong *et al* (1976) have studied the largest number of individuals. In their series, the birth weight of 10 608 (Mean±SD) male and 10 043 female Yoruba children in a non-teaching hospital at Oluyore near Ibadan was 3·00±0·51 kg and 2·88±0·49 kg respectively. Both these figures are significantly lower than ours (P <0·001). Similarly their figures of 2·98±0·56 kg for 5622 males and 28·6±0·54 kg for 5217 females at University College Hospital, Ibadan were also significantly lower than our figures (P <0·001). The difference may reflect a number of differences in the populations studied. Whereas our series includes only the spontaneous normal deliveries, their series covered both normal and complicated cases. Although they state that the 'majority' of deliveries at the rural hospital were normal, yet the fact that the data include both singleton and multiple births could account at least in part for a lower mean birth weight in an area where the incidence of twinning is 53 per 1000 births (Knox and Morley, 1960). Moreover, there is no information about the gestational age of these newborn infants and if preterm deliveries were also included this too would reduce the mean birth weight.

The mean birth weight of 1290 predominantly Yoruba children reported by Ladipo and Adelusi (1977) was 3·15 kg for males and 2·95 for females. Unfortunately they did not give any measure of the variation so it is difficult to make an appropriate comparison.

Osuthor (1976) reported the birth weight of 1199 babies in Malumfashi, about 150 km from our hospital. He gave the mean birth weight of 627 Hausa children as 2·85 kg; this is much below our figure, but he also gave no indication of variation and a comparative analysis is not possible. But his data included preterm deliveries, stillbirths and other abnormalities which might account for a lower mean birth weight.

Unfortunately none of the above studies calculated the birth weight for given weeks of gestation so we are not able to compare our findings on that basis.

Brueton (1975) made a study at Zaria, about 280 km from us, to assess the gestational age of northern Nigerian children. That study shows the mean birth weights of 850 children of 37 to 41 weeks gestation to be almost identical to ours. Although he did not calculate the overall mean birth weight, making further calculations on his figures gives a mean birth weight of $3·00 \pm 0·48$ (SD) kg. This figure closely approximates our figure and there is no significant difference between the two. The birth weight (Mean ± SD) for 453 males in his series was $3·06 \pm 0·66$ kg and for 397 females $2·93 \pm 0·40$ kg. Both these figures do not differ significantly from ours.

The comparison of our figures with the reports from other countries shows that the mean birth weight in our study was significantly lower than of Jamaican children (Roopanarainsingh *et al*, 1971) as well as of American white and negro children (Anderson *et al*, 1943). The comparison of our data on a gestation-specific basis with the corresponding figures from the Jamaican study shows that at each week of gestation Jamaican babies of both sexes are heavier than their Hausa counterparts. Similarly, in comparing our figures with the mean birth weight of Scottish babies (Thomson *et al*, 1968), we find that Hausa children are not only lighter at each week of gestation, but their intrauterine growth ceases earlier. The mean birth weight of Indian boys and girls as reported by Thomson (1962) is significantly lower than their Hausa counterparts ($P < 0·001$), whereas the mean birth weight of Chinese boys and girls in the same series was significantly higher than the corresponding figures in our study. Lewis (1974) found the mean birth weight of 807 singleton children in Cameroon to be 3·119 kg, but this figure in the absence of other information does not allow an appropriate comparison.

Our data agree with those of Thomson *et al* (1968) and Ounsted (1974) that the second and subsequent babies are heavier than the first born. However, our findings do not support the suggestion of Selvin and Janerich (1971) that increasing birth order for mothers under 20 years is associated with a decrease in birth weight. In our series increasing birth order was associated with heavier birth weight irrespective of maternal age (Table IV). Our data do not agree with the views expressed by Thomson *et al* (1968) and Barron (1974) that there is no increase in the birth weight of children after the second child. In our series there was a significant increase in the birth weight to the fourth child. The most likely explanation may be that since the women in this area start child bearing before they are fully grown, their reproductive faculties —anatomical as well as physiological—are not fully developed during the first few pregnancies. Similar findings have also been reported by Camillera and Cremona (1970) from Malta, who found a convincing progression of birth weight with increasing parity up to ten.

The overall incidence of low birth weight was 15·8 per cent. Ladipo and Adelusi (1977) reported the incidence of low birth weight as 24·9 per cent for University College, Ibadan, whereas Effiong *et al* (1976) working from the same hospital found the incidence to be 19·3 per cent. On the other hand Oduntan and Ayeni

(1976) who also carried out their study in Ibadan report an incidence of only 9·4 per cent. It is very difficult to pinpoint the factors responsible for differences in their figures, but the data of Ladipo and Adelusi (1977), and of Effiong *et al* (1975) include a fairly large proportion (15 per cent and 39 per cent respectively) of preterm deliveries. The same observations apply to the findings of Osuthor (1976), who reported an incidence of low birth weight of 20 per cent in Malumfashi. If all preterm deliveries are excluded from the data of Ladipo and Adelusi (1977), the incidence of low birth weight becomes 13 per cent, which compares favourably with our figure.

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BIOCHEMICAL CHANGES OF NORMAL PREGNANCY AND PUERPERIUM IN LAGOS, NIGERIA

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Summary

A cross-sectional study is presented of biochemical changes in the third trimester of normal pregnancy and puerperium during the wet season in the tropical climate of Lagos, Nigeria. These changes are less marked in first than in subsequent pregnancies, and although qualitatively similar, in some respects they differ from those observed in the temperate climatic zone. The indications are that quantitative differences may exist which could be relevant to the management of pregnancy in the tropics.

THE majority of studies on the biochemical changes of normal pregnancy have been carried out in temperate climate (Hytten and Lind, 1973) and there is little corresponding information about pregnancy in the tropics. We therefore present an evaluation of biochemical changes in the third trimester of normal pregnancy and puerperium during the wet season in Lagos, Nigeria.

METHODS

The control subjects were 51 non-pregnant women, of whom 31 were nulliparous. They were drawn from hospital nursing, administrative and ancillary staff, and school girls. Women with cardiac disease, respiratory disease, nephritis and hypertension were excluded, as were women on any form of oral contraception and those who were menstruating at the time.

The 60 pregnant subjects, including 25 primigravidae, were in the third trimester of pregnancy and were attending the antenatal clinics of Lagos University Teaching Hospital and Lagos Island Maternity Hospital during May and June 1977. None suffering from any of the disorders listed above was included.

Thirty-five women who had normal pregnancies and deliveries at term were also investigated from 0 to 6 weeks post-partum; eight were primiparae.

No ethnic group selection was applied and the sample population included representatives of the major tribes resident in the city.

As far as possible, investigations were done between 1030 and 1400 hours and the subjects had nothing to eat or drink less than two hours before investigation.

The 20 ml blood samples were drawn from the antecubital vein using a tourniquet, transferred to lithium-heparin containers and the plasma was separated within two hours of collection. Two ml volumes of plasma were used for osmolality determination by the Advanced Osmometer, Model 3L, while the remainder was used for the determination of sodium, potassium, chloride, bicarbonate, total protein, albumin, total calcium, cholesterol, urea nitrogen, uric acid, creatinine and glucose by the Technicon Multichannel Auto-Analyser, Model SMA 12/60.

To facilitate direct comparison with earlier work, the results are expressed in common units, but the following factors may be used to convert the data to SI units if required. For sodium, potassium, chloride and bicarbonate the factor is 1·0; for total protein and albumin, 10; total calcium, 0·25; cholesterol, 0·0259; urea nitrogen, 0·357; uric acid, 0·0598; creatinine, 88·4 and glucose, 0·0555.

The blood pressures were recorded with the subjects comfortably seated upright. The point of muffling of the sound (phase IV) was read as the diastolic pressure and the mean arterial pressure was calculated from the measured values. Usually blood pressure measurement and blood sample collection were within 5 minutes of each other.

RESULTS

The data on 31 non-pregnant nulliparous women and 25 third trimester primigravidae are given in Table I. There was no significant difference in age, plasma sodium, potassium, chloride, cholesterol, uric acid, creatinine, osmolality or mean arterial pressure, but in late pregnancy levels of plasma bicarbonate ($P < 0{\cdot}05$), total protein ($P < 0{\cdot}001$), albumin ($P < 0{\cdot}001$), total calcium ($P < 0{\cdot}001$), urea ($P < 0{\cdot}001$) and glucose ($P < 0{\cdot}05$) were significantly lower.

The data on 20 non-pregnant parous women and 35 third trimester multigravidae are recorded in Table II. The changes associated with pregnancy were more extensive, with significant differences in 12 of the 14 measured variables. Their mean ages were not significantly different,

TABLE I

Biochemical changes in the third trimester of normal primigravidae

Measured variable	Non-pregnant nulliparous (n = 31) Mean ± SEM	Third trimester primigravidae (n = 25) Mean ± SEM	Difference of means	t value	Statistical significance
Age (years)	21·1 ± 0·7	20·1 ± 0·6	1·0	1·0846	NS
Sodium (meq/l)	139·7 ± 0·3	138·9 ± 0·6	0·8	1·1926	NS
Potassium (meq/l)	3·71 ± 0·06	3·75 ± 0·07	0·04	0·4338	NS
Chloride (meq/l)	100·0 ± 1·1	103·2 ± 1·7	3·2	1·5804	NS
Bicarbonate (meq/l)	25·0 ± 0·4	23·2 ± 0·7	1·8	2·2327	$P < 0{\cdot}05$
Total protein (g/dl)	7·91 ± 0·11	7·00 ± 0·16	0·91	4·6859	$P < 0{\cdot}001$
Albumin (g/dl)	3·94 ± 0·08	3·52 ± 0·05	0·42	4·4539	$P < 0{\cdot}001$
Total calcium (mg/dl)	9·11 ± 0·08	8·44 ± 0·13	0·67	4·3906	$P < 0{\cdot}001$
Cholesterol (mg/dl)	169·4 ± 5·9	184·5 ± 10·6	15·1	1·2447	NS
Urea nitrogen (mg/dl)	9·55 ± 0·61	6·64 ± 0·51	2·91	3·6599	$P < 0{\cdot}001$
Uric acid (mg/dl)	4·15 ± 0·13	4·30 ± 0·21	0·15	0·6073	NS
Creatinine (mg/dl)	0·91 ± 0·04	0·86 ± 0·04	0·05	0·8834	NS
Glucose (mg/dl)	94·6 ± 2·4	87·9 ± 2·3	6·7	2·0155	$P < 0{\cdot}05$
Plasma osmolality (mosmol/kg H_2O)	275·6 ± 2·4	274·1 ± 2·3	1·5	0·4512	NS
Mean arterial pressure (mm Hg)	80·5 ± 1·5	80·8 ± 1·8	0·3	0·1280	NS

TABLE II

Biochemical changes in the third trimester of normal multigravidae

Measured variable	Non-pregnant parous (n = 20) Mean ± SEM	Third trimester multigravidae (n = 35) Mean ± SEM	Difference of Means	t value	Statistical significance
Age (years)	29·5 ± 1·2	28·5 ± 0·9	1·0	0·6667	NS
Sodium (meq/l)	139·3 ± 0·5	138·0 ± 0·4	1·3	2·0303	P < 0·05
Potassium (meq/l)	3·79 ± 0·09	3·60 ± 0·07	0·19	1·6667	NS
Chloride (meq/l)	98·7 ± 1·0	102·5 ± 1·1	3·8	2·5562	P < 0·02
Bicarbonate (meq/l)	26·1 ± 0·5	23·2 ± 0·4	2·9	4·5291	P < 0·001
Total protein (g/dl)	7·91 ± 0·16	6·74 ± 0·08	1·17	6·5400	P < 0·001
Albumin (g/dl)	3·96 ± 0·07	3·29 ± 0·06	0·67	7·2668	P < 0·001
Total calcium (mg/dl)	9·09 ± 0·13	8·63 ± 0·08	0·46	3·0144	P < 0·005
Cholesterol (mg/dl)	166·7 ± 8·5	190·1 ± 5·3	23·4	2·3360	P < 0·025
Urea nitrogen (mg/dl)	10·40 ± 0·70	6·03 ± 0·29	4·37	5·7675	P < 0·001
Uric acid (mg/dl)	4·50 ± 0·20	3·84 ± 0·14	0·66	2·7038	P < 0·01
Creatinine (mg/dl)	1·03 ± 0·05	0·83 ± 0·03	0·20	3·4305	P < 0·005
Glucose (mg/dl)	93·0 ± 2·8	90·3 ± 2·8	2·7	0·6819	NS
Plasma osmolality (mosmol/kg H_2O)	281·2 ± 2·1	272·6 ± 2·0	8·6	4·3000	P < 0·001
Mean arterial pressure (mm Hg)	90·7 ± 2·6	81·2 ± 1·3	9·5	3·2681	P < 0·005

TABLE III

Biochemical changes associated with the transition from pregnancy to the puerperium in normal primiparae

Measured variable	Third trimester primigravidae (n = 25) Mean ± SEM	Puerperal primiparae (n = 8) Mean ± SEM	Difference of Means	t value	Statistical significance
Age (years)	20·1 ± 0·6	22·3 ± 1·6	2·2	1·2875	NS
Sodium (meq/l)	138·9 ± 0·6	137·8 ± 0·6	1·1	1·2964	NS
Potassium (meq/l)	3·75 ± 0·07	3·36 ± 0·08	0·39	3·6689	P < 0·001
Chloride (meq/l)	103·2 ± 1·7	107·9 ± 1·5	4·7	2·0730	P < 0·05
Bicarbonate (meq/l)	23·2 ± 0·7	24·1 ± 1·4	0·9	0·5750	NS
Total protein (g/dl)	7·00 ± 0·16	6·60 ± 0·27	0·40	1·2747	NS
Albumin (g/dl)	3·52 ± 0·05	3·62 ± 0·13	0·10	0·7179	NS
Total calcium (mg/dl)	8·44 ± 0·13	8·69 ± 0·21	0·25	1·0121	NS
Cholesterol (mg/dl)	184·5 ± 10·6	188·0 ± 24·7	3·5	0·1302	NS
Urea nitrogen (mg/dl)	6·64 ± 0·51	10·63 ± 0·71	3·99	4·5642	P < 0·001
Uric acid (mg/dl)	4·30 ± 0·21	5·25 ± 0·42	0·95	2·0230	0·10 > > P > 0·05
Creatinine (mg/dl)	0·86 ± 0·04	1·15 ± 0·08	0·29	3·2438	P < 0·005
Plasma osmolality (mosmol/kg H_2O)	274·1 ± 2·3	291·8 ± 6·9	17·7	2·4336	P < 0·025
Mean arterial pressure (mm Hg)	80·8 ± 1·8	92·0 ± 5·0	11·2	2·1076	P < 0·05

nor were the potassium and glucose levels. In the pregnant women there was an increase in both plasma chloride (P < 0·02) and cholesterol (P < 0·025) but there were reductions in plasma sodium (P < 0·05), bicarbonate (P < 0·001), total protein (P < 0·001), albumin (P < 0·005), total calcium (P < 0·005), urea (P < 0·001), uric acid (P < 0·01), creatinine (P < 0·005), osmolality (P < 0·001) and mean arterial pressure (P < 0·005).

The comparison of the data for third trimester pregnancies with those of the puerperium is shown in Table III for primiparae.

There was no significant difference in age, plasma sodium, bicarbonate, total protein, albumin, total calcium, cholesterol, or uric acid.

TABLE IV

Biochemical changes associated with the transition from pregnancy to the puerperium in normal multiparae

Measured variable	Third trimester multigravidae (n = 35) Mean ± SEM	Puerperal multiparae (n = 27) Mean ± SEM	Difference of Means	t value	Statistical significance
Age (years)	28·5±0·9	25·8±1·1	2·7	1·8997	NS
Sodium (meq/l)	138·0±0·4	137·9±0·4	0·1	0·1768	NS
Potassium (meq/l)	3·60±0·07	3·47±0·07	0·13	1·3131	NS
Chloride (meq/l)	102·5±1·1	107·2±0·8	4·7	3·4556	P <0·005
Bicarbonate (meq/l)	23·2±0·4	24·8±0·8	1·6	1·7889	NS
Total protein (g/dl)	6·74±0·08	6·97±0·15	0·23	1·3529	NS
Albumin (g/dl)	3·29±0·06	3·84±0·05	0·55	7·0423	P <0·001
Total calcium (mg/dl)	8·63±0·08	8·92±0·10	0·29	2·2639	P <0·05
Cholesterol (mg/dl)	190·1±5·3	186·2±5·5	3·9	0·5106	NS
Urea nitrogen (mg/dl)	6·03±0·29	8·93±0·54	2·90	4·7316	P <0·001
Uric acid (mg/dl)	3·84±0·14	5·40±0·20	1·56	6·3908	P <0·001
Creatinine (mg/dl)	0·83±0·03	1·11±0·02	0·28	7·7562	P <0·001
Plasma osmolality (mosmol/kg H_2O)	272·6±2·0	295·6±3·0	23·0	6·3790	P <0·001
Mean arterial pressure (mm Hg)	81·2±1·3	92·3±1·8	11·1	4·9991	P <0·001

TABLE V

Biochemical changes associated with the reversion to the non-pregnant state in normal primiparae

Measured variable	Puerperal primiparae (n = 8) Mean ± SEM	Non-pregnant primiparae (n = 6) Mean ± SEM	Difference of Means	t value	Statistical significance
Age (years)	22·3±1·6	25·0±1·1	2·7	1·3906	NS
Sodium (meq/l)	137·8±0·6	140·3±0·6	2·5	2·9464	P <0·02
Potassium (meq/l)	3·36±0·08	3·67±0·13	0·31	2·0315	NS
Chloride (meq/l)	107·9±1·5	100·3±1·5	7·6	3·5820	P <0·005
Bicarbonate (meq/l)	24·1±1·4	26·7±0·8	2·6	1·6124	NS
Total protein (g/dl)	6·60±0·27	7·88±0·40	1·28	2·6523	P <0·025
Albumin (g/dl)	3·62±0·13	4·00±0·14	0·38	4·1758	P <0·005
Total calcium (mg/dl)	8·69±0·21	9·07±0·21	0·38	1·2795	NS
Cholesterol (mg/dl)	188·0±24·7	142·5±9·8	45·5	1·7123	NS
Urea nitrogen (mg/dl)	10·63±0·71	10·17 ±1·1	0·46	0·3514	NS
Uric acid (mg/dl)	5·25±0·42	4·28±0·34	0·97	1·7950	NS
Creatinine (mg/dl)	1·15±0·08	1·00±0·11	0·15	1·1029	NS
Plasma osmolality (mosmol/kg H_2O)	291·8±6·9	283·2±3·6	8·6	1·1050	NS
Mean arterial pressure (mm Hg)	92·0±5·0	86·8±4·3	5·2	0·7885	NS

Plasma potassium was significantly lower (P <0·001) in the puerperium, whereas plasma chloride (P <0·05), urea (P <0·001), creatinine (P <0·005), osmolality (P <0·025) and mean arterial pressure (P <0·05) were significantly higher.

The same comparison for multiparae (Table IV) indicates that neither age nor plasma sodium, potassium, bicarbonate, total protein and cholesterol were significantly different. In the puerperium there was a significant increase in plasma chloride (P <0·005), albumin (P <0·001), total calcium (P <0·05), urea (P <0·001), uric acid (P <0·001), creatinine (P <0·001), osmolality (P <0·001), and mean arterial pressure (P <0·001).

Primiparous women in the puerperium are compared with those who had not recently been pregnant in Table V. Plasma chloride was significantly lower (P <0·005), while significantly higher levels of sodium (P <0·02), total protein (P <0·025) and albumin (P

TABLE VI

Biochemical changes associated with the reversion to the non-pregnant state in normal multiparae

Measured variable	Puerperal multiparae (n = 27) Mean ± SEM	Non-pregnant multiparae (n = 14) Mean ± SEM	Difference of Means	t value	Statistical significance
Age (years)	25·8 ± 1·1	31·4 ± 1·4	5·6	3·1454	$P < 0{\cdot}005$
Sodium (meq/l)	137·9 ± 0·4	138·8 ± 0·6	0·9	1·2481	NS
Potassium (meq/l)	3·47 ± 0·07	3·84 ± 0·11	0·37	2·8374	$P < 0{\cdot}01$
Chloride (meq/l)	107·2 ± 0·8	98·0 ± 1·3	9·2	6·0273	$P < 0{\cdot}001$
Bicarbonate (meq/l)	24·8 ± 0·8	25·8 ± 0·7	1·0	0·9407	NS
Total protein (g/dl)	6·97 ± 0·15	7·91 ± 0·16	0·94	4·2864	$P < 0{\cdot}001$
Albumin (g/dl)	3·84 ± 0·05	3·94 ± 0·08	0·10	1·0604	NS
Total calcium (mg/dl)	8·92 ± 0·10	9·10 ± 0·16	0·18	0·9539	NS
Cholesterol (mg/dl)	186·2 ± 5·5	177·1 ± 10·4	9·1	0·7735	NS
Urea nitrogen (mg/dl)	8·93 ± 0·54	10·50 ± 0·91	1·57	1·4022	NS
Uric acid (mg/dl)	5·40 ± 0·20	4·59 ± 0·25	0·81	2·5297	$P < 0{\cdot}02$
Creatinine (mg/dl)	1·11 ± 0·02	1·04 ± 0·06	0·07	1·1076	NS
Plasma osmolality (mosmol/kg H_2O)	295·6 ± 3·0	280·4 ± 2·7	15·2	3·7660	$P < 0{\cdot}001$
Mean arterial pressure (mm Hg)	92·3 ± 5·0	92·5 ± 3·2	0·2	0·0337	NS

$< 0{\cdot}005$) occurred in primiparae who were past the puerperium, but there are no other significant differences.

With multiparae (Table VI) the same comparison indicates that multiparous women who were past the puerperium had a significantly higher plasma potassium ($P < 0{\cdot}01$) and total protein ($P < 0{\cdot}001$), but significantly lower chloride ($P < 0{\cdot}001$), uric acid ($P < 0{\cdot}02$) and osmolality ($P < 0{\cdot}001$).

Some of the observed changes are summarized graphically in Figures 1 and 2.

DISCUSSION

The data presented here are cross-sectional, and thus refer to separate groups of women at the different stages during the two-month period of study. They are, however, more comprehensive than any previously reported, indicating the biochemical changes in normal pregnancies terminating at the height of the Nigerian wet season, and the biochemical distinction between first and subsequent pregnancies. Some of the results reported here differ from other published data, due perhaps partly to the parity distinction, but maybe also to climatic dietetic and ethnic factors. Nevertheless, the observed trends are broadly in agreement with those recorded in the critical data compilation of Hytten and Lind (1973).

Plasma sodium tended to be lower in late pregnancy irrespective of parity, and while bicarbonate also fell due to the compensated respiratory alkalosis, chloride rose as a result of the chloride shift. The reduction in total protein and albumin in both parity groups was in agreement with the observations of Robertson and Cheyne (1972).

The fall in total calcium was not unexpected, because about 50 per cent of plasma calcium is albumin-bound and there is evidence (Tan *et al*, 1972) that the ionized fraction is also reduced. Excepting chloride, cholesterol was the only constituent that increased in pregnancy and while this observation is in agreement with other studies (Hytten and Lind, 1973) it is unexplained. Urea nitrogen exhibited the greatest change, falling by about 30 per cent in primigravidae and 40 per cent in multigravidae. This may be partly due to the increased glomerular filtration rate (Hytten and Lind, 1973) and the increase in total body water, for urea is passively distributed throughout the body fluids. Plasma uric acid, creatinine and glucose also tended to fall, but the changes are much smaller than for urea. The glomerular filtration rate change may account for the uric acid and creatinine level in pregnancy. With glucose less effective renal resorption may be relevant (Davison, 1975). The decrease in plasma osmolality here was much smaller in primigravidae, and thus differed from Robertson and Cheyne (1972), who reported a

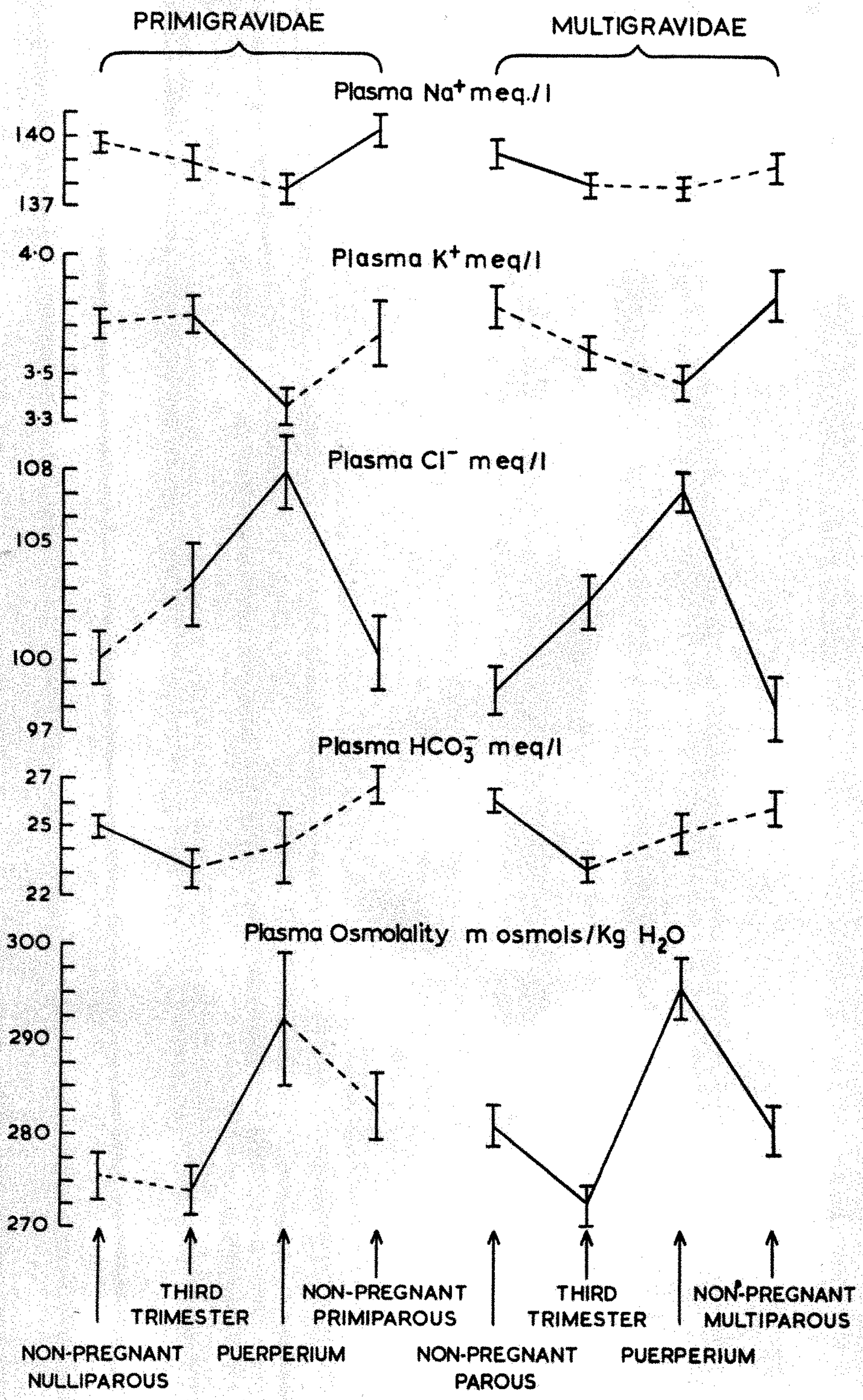

FIG. 1

The changes in plasma sodium, potassium, chloride bicarbonate and osmolality associated with pregnancy and the puerperium.

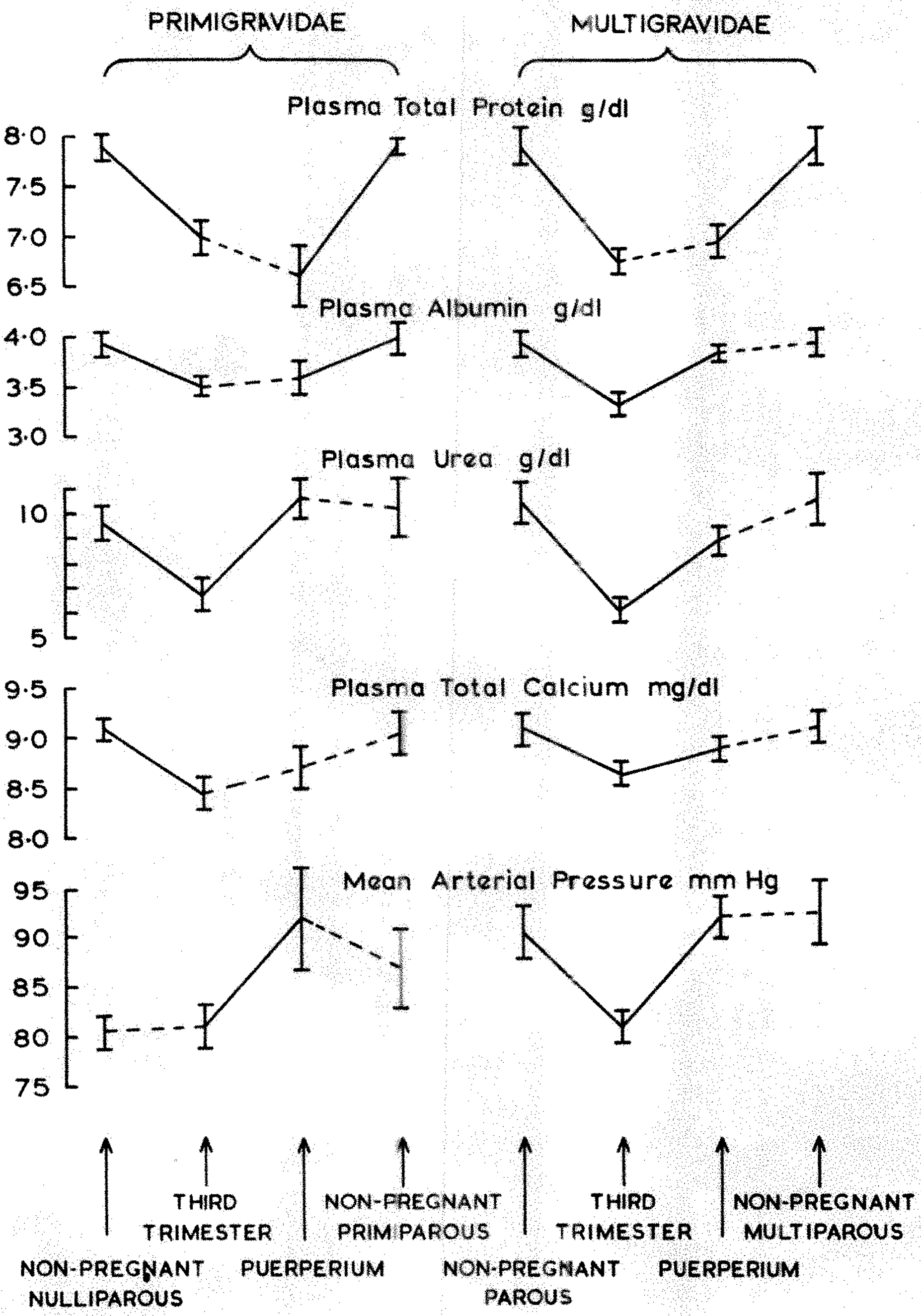

FIG. 2

The changes in plasma total protein, albumin, urea nitrogen, total calcium and mean arterial pressure associated with pregnancy and the puerperium.

fall of about 9 mosmol, similar to that of our multigravidae, in a predominantly primigravid population. It is also noteworthy that the plasma osmolality in non-pregnant women in Nigeria, was significantly lower ($P < 0{\cdot}001$) than that of non-pregnant women in England ($289 \pm 0{\cdot}5$ mosmol; Robertson and Cheyne, 1972), or a mixed group of male and non-pregnant female subjects in Scotland ($289{\cdot}0 \pm 0{\cdot}6$ mosmol; Hendry, 1961). The mean non-pregnant level of plasma osmolality in Nigeria is, in fact, similar to that for late pregnancy in England. The mean arterial pressure (MAP) was apparently unchanged with first pregnancies, but was significantly higher ($P < 0{\cdot}005$) in non-pregnant multiparae and was 10 per cent lower in pregnant multigravidae. The former result is in keeping with the observations of MacGillivray *et al* (1969), who found that the MAP (sitting) of English primigravidae near term was around 80 mm Hg, with a minimum value of about 70 mm Hg early in the second trimester, but the latter has not previously been reported.

The transition from pregnancy to puerperium is accompanied by marked changes in plasma solute concentrations which vary considerably with different constituents and time of sampling (Macdonald *et al*, 1974) and although that report refers to data obtained in England, the corresponding changes which occur in Nigeria are probably also highly dependent on the time of sampling. Despite that, however, and the additional disadvantage of cross-sectional investigation, several of the measured variables exhibited consistent and significant differences.

The major puerperal change was the increase in plasma osmolality, around 18 mosmol in primiparae and 23 mosmol in multiparae; the former is twice the corresponding value for primiparae in England (Robertson and Cheyne, 1972; Macdonald *et al*, 1974).

Most plasma solutes were also higher in the puerperium, particularly in multiparae. The cholesterol level was unchanged, thus implying that the cholesterol content is independently reduced. Surprisingly, sodium was also unchanged, while potassium tended to be lower. These observations contrast with those of Macdonald *et al* (1974) which, despite the smaller increase in plasma osmolality, demonstrated highly significant rises in both ion species within 72 hours of parturition. The reason for this discrepancy is not clear and a similar cross-sectional study in the dry season might indicate the extent to which climatic factors are involved.

The reversion from the puerperium to the non-pregnant state was accompanied by a substantial reduction in plasma osmolality—or increase in plasma water content—in both parity groups, with the change almost twice as great in primiparae. Despite this, and the consequently lower plasma viscosity, there was no significant difference in mean arterial pressure, which appears to remain elevated after the first pregnancy. The trends of the plasma solutes generally were a partial reversal of the changes incurred during the transition from pregnancy to the puerperium. Thus the discrepancies noted in that context are equally relevant here, for despite plasma dilution, sodium, potassium, bicarbonate and protein concentrations were raised. It is clear, therefore, that a great deal remains to be discovered about the transition from pregnancy to the non-pregnant state.

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THE RELATION BETWEEN PREGNANCY SPECIFIC β_1 GLYCOPROTEIN (SP_1) LEVELS AND ABNORMAL GLUCOSE TOLERANCE IN PREGNANCY

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Summary

Pregnancy specific β_1 glycoprotein (SP_1) levels were measured in 54 potentially diabetic patients in the third trimester of pregnancy using a laser nephelometric method. All patients were tested by the 50 g oral glucose tolerance test. The results show that a significantly greater number of patients with abnormal glucose tolerance had SP_1 levels above the mean compared with patients with normal glucose responses. All patients with a diabetic type response had SP_1 levels above the mean. There was no significant difference in SP_1 levels in the fasting and two-hour plasma samples. The study suggests that patients with asymptomatic glucose intolerance in pregnancy have higher SP_1 levels than patients with normal glucose tolerance. It is possible that one of the factors controlling the placental output of SP_1 is the maternal blood glucose level.

PREGNANCY specific β_1 glycoprotein (SP_1) has been shown by Tatra *et al* (1976) to be significantly increased 24 hours after a hypo- or hyperglycaemic episode. They concluded that SP_1 levels and carbohydrate metabolism might be inter-related. It has also been shown that levels of this protein increase as pregnancy progresses and obviously any placental substance which behaves in this manner will stimulate curiosity in relation to glucose tolerance which deteriorates with increasing gestation.

In view of the continuing controversy as to the reasons for the alterations in glucose tolerance in pregnancy it seemed appropriate to assess the relationship of SP_1 levels to carbohydrate tolerance.

The aim of our study was to determine whether SP_1 levels in patients with asymptomatic glucose intolerance in pregnancy were different to those in patients with normal glucose tolerance.

We felt that asymptomatic glucose intolerance was a term which was more descriptive of the metabolic alterations than chemical gestational diabetes.

PATIENTS AND METHODS

Patients for the study were selected from those

TABLE I

Indications for oral glucose tolerance test

Glycosuria (second fasting specimen)
Family history of diabetes
Maternal weight for height above the 85th centile
Previous baby with birth weight above 95th centile
Previous unexplained stillbirth or neonatal death
Previous congenital abnormality
Presence of hydramnios

attending the Maternity Unit at the Southampton General Hospital for oral glucose tolerance tests (OGTTs). The selection criteria for OGTTs are given in Table I. A standard 50 g oral glucose tolerance test was performed after an overnight fast of at least 10 hours. Venous blood samples (fasting and half-hourly up to 2½ hours after glucose ingestion) were obtained. Aliquots from the fasting and two-hour samples were centrifuged and deep frozen within three hours of collection and stored for SP_1 analysis.

Plasma glucose estimations were performed in the routine service laboratory using the glucose-oxidase/peroxidase system on an auto-analyser. The criteria adopted for abnormality of the oral glucose tolerance test were based on the recommendations of the WHO Expert Committee (1965). A two-hour plasma glucose of between 6·11 to 7·15 mmol/l (110 to 129 mg/dl) was considered borderline and a diabetic state was said to exist if levels were 7·16 mmol/l (129 mg/dl) or more.

A laser nephelometric method was used to determine SP_1 levels in maternal plasma (Wood *et al*, 1978). SP_1 standard and rabbit anti-human-SP_1-serum were purchased from Behringwerke AG. The assay buffer consisted of a 0·05

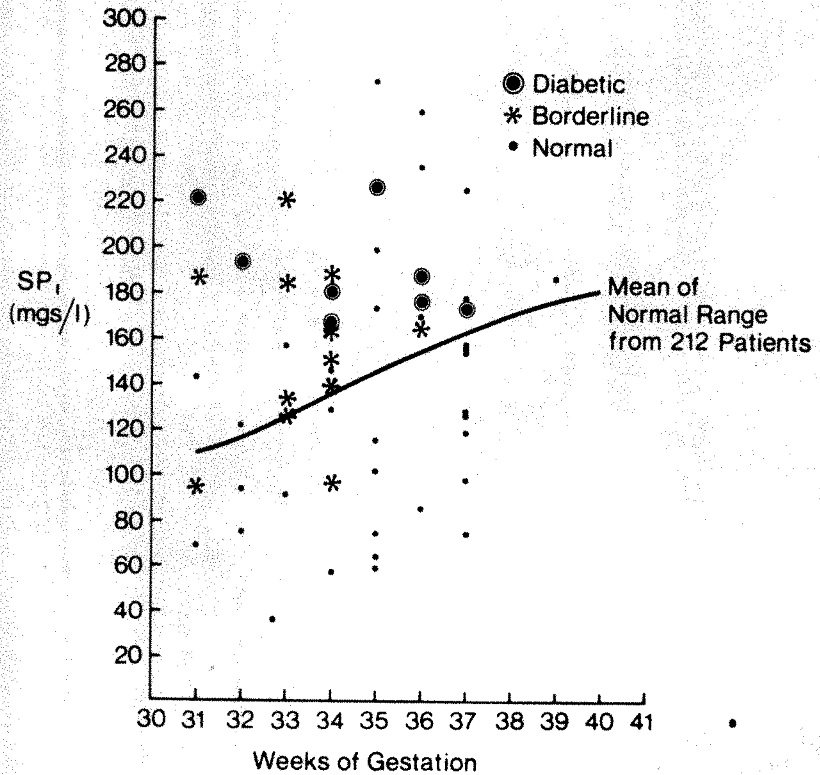

FIG. 1

Distribution of fasting pregnancy specific β_1 glycoprotein (SP_1) levels in the 54 patients studied. The values are superimposed on the mean of the range established by Wood *et al* (1978).

mmol/l phosphate buffer, pH 7·4, containing sodium chloride (0·1 mmol/l) and polyethylene glycol 6000 (40 g/l). Aliquots (50 μl) of plasma samples or standards were mixed with 500 μl of a 1 in 5 dilution of protamine sulphate solution (10 g/l) in assay buffer. After centrifugation, 50 μl aliquots of the supernatant solutions were added to 1·0 ml of assay buffer (for blanks) or to 1·0 ml of a 1 in 200 dilution of neat antiserum in assay buffer (for tests) in Hyland laser nephelometer cuvettes. The solutions were vortexed and incubated for one hour at 37°C. The relative light scatter readings were determined after incubation in the Hyland 'PDQ' laser nephelometer. Test and standard results were corrected for individual 'blank' relative light scatter reading.

The mean plasma SP_1 concentrations established by Wood *et al* (1978) on samples from 212 normal patients taken between 31 and 41 weeks gestation were used as reference for interpretation of our results. The birth weights of all their babies were between the 10th and 90th centile and the graph for the mean SP_1 values given by Wood *et al* (1978) appears in Figure 1.

For the conversion of birth weights and placental weights into centiles, reference tables published by Thomson *et al* (1968) were used for birth weights and those published by Kloosterman (1970) for placental weights.

RESULTS

The distribution of results of OGTT is shown in Table II. Of the 54 patients tested, 34 gave a normal response, 12 a borderline and 8 a diabetic type response. The 15 per cent incidence of a diabetic response is comparable to the incidence reported previously by Singh and Arshat (1978).

TABLE II

Distribution of oral glucose tolerance test results in late pregnancy

Patient groups	Number	per cent
Normal	34	63
Borderline	12	22
'Diabetic'	8	15
	54	100

When the fasting and two-hour SP_1 values were analysed no significant differences were noted and we concluded that SP_1 levels were uninfluenced by short term variations in glucose concentration.

Figure 1 shows the distribution of fasting SP_1 levels from the 54 patients studied. The values are superimposed on the mean curve established by Wood *et al* (1978). There is a wide scatter of SP_1 values in patients with normal OGTTs. The majority of the patients with borderline responses were above the mean with only two patients below the mean, and all eight patients with diabetic type responses showed values above the mean.

SP_1 results in the three patient groups are given in Table III. The differences in numbers above and below the mean are all statistically

TABLE III

Incidence of pregnancy specific β_1 glycoprotein (SP_1) values above and below the mean in patients with normal, borderline and diabetic oral glucose tolerance tests

	No. of patients with SP_1 values	
Patient group	Below mean	Above mean
Normal (N)	21	13
Borderline (B)	2	10
'Diabetic' (D)	0	8

Fisher's exact test: N *vs* B $p<0·01$; N+B *vs* D $p<0·01$; B *vs* D p = NS; N *vs* D+B $p<0·001$.

TABLE IV

Distribution of birth weights above and below 50th centile in patients with normal, borderline and diabetic oral glucose tolerance tests

	No. of patients with birth weights	
Patient group	Below 50th centile	Above 50th centile
Normal (N)	13	21
Borderline (B)	6	6
Diabetic (D)	4	4

Fisher's exact test showed no significant differences: N *vs* B; N+B *vs* D; B *vs* D; N *vs* D+B.

TABLE V

Distribution of placental weights above, and below 50th centile in patients with normal, borderline and diabetic oral glucose tolerance tests

Patient group	No. of patients with placental weights	
	Below 50th centile	Above 50th centile
Normal (N)	5	27
Borderline (B)	3	9
Diabetic (D)	1	6

Fisher's exact test showed no significant differences: N *vs* B; N+B *vs* D; B *vs* D; N *vs* D+B.

significant except when the borderline and diabetic groups were compared. In view of the possibility that the SP_1 levels may be a reflection of birth weight and placental weight rather than circulating plasma glucose levels, the birth weight and placental weight data were examined in the three groups.

No significant differences in the number of babies above and below the 50th centile for birth weights was found (Table IV). A similar observation was made when the centile placental weights were assessed. But there was a bias towards greater numbers with higher placental weights (Table V).

There were no stillbirths or neonatal deaths in the group studied.

DISCUSSION

Tatra *et al* (1976) have shown that 24 hours after the intravenous administration of insulin or glucose, SP_1 levels were significantly increased. The implication is that glucose levels may in some way be associated with SP_1 levels. In our study, the fact that there were no significant differences in the fasting and two-hour plasma SP_1 levels would suggest that short term variations in glucose levels do not alter SP_1 concentration. These findings are in agreement with those of Tatra *et al* (1976) who also showed little short term change in SP_1 levels on intravenous glucose loading.

We have demonstrated that a significantly greater number of patients with elevated two-hour plasma glucose levels in response to OGTT have high SP_1 levels compared with those who demonstrated normal responses.

Although these observations would suggest that SP_1 levels are controlled by circulating glucose levels, it is not unreasonable to suppose that SP_1 may, like human placental lactogen (HPL), be antagonistic to the action of insulin and may indeed be responsible for the abnormal glucose tolerance.

There is controversy about the claim that HPL is the diabetogenic hormone of pregnancy. Spellacy *et al* (1971) showed that there were no changes in HPL levels following glucose infusion, whereas Pavlov *et al* (1972) reported a statistically significant fall in HPL levels following glucose administration. More recently Singh and Arshat (1978) have shown a significant though low negative correlation between HPL and the two-hour plasma glucose of the OGTT.

Our results show that there were no significant differences between the birth weights and placental weights in the three groups and the suggestion therefore is that the differences in SP_1 levels are in some way associated with circulating blood glucose levels or with a diabetic state. It would be erroneous, however, to postulate that maternal plasma glucose and SP_1 levels are inter-related to the exclusion of other factors, for example non-esterified fatty acids, and ketone bodies. Further work to assess the relationship of these insulin antagonists and SP_1 is in progress.

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DIFFERENCES IN ALBUMIN DISTRIBUTION AND DYNAMICS BETWEEN TOXAEMIC AND NON-TOXAEMIC PREGNANT WOMEN

BY

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Summary

By using least squares curve fitting techniques, double exponential curves were fitted to points representing the decay of plasma ^{131}I-albumin after blood volume estimation in 48 pregnant women. Data from the fitted curves provided estimates of the distribution of albumin between plasma and extravascular compartments, exchange between the two and rate of albumin catabolism. The lower total extracellular albumin mass of a group of 23 severely toxaemic patients was shown to arise solely from the difference in plasma albumin mass between this group and the remaining 25 patients. Comparison of these results with those in the literature suggests that pregnant women have a higher proportion of their exchangeable albumin mass in the plasma compartment and a higher rate of albumin catabolism than non-pregnant women.

SERUM albumin concentration in patients with toxaemia of pregnancy is known to be reduced below the normally low serum albumin of pregnancy (Hytten and Lind, 1973). In view of the oedema frequently associated with toxaemia it is pertinent to consider whether there is an overall reduction in extracellular albumin in women with this condition or whether it is the disposition which is at fault with plasma albumin leaked to the interstitial fluid. The present report describes studies in 48 pregnant women, including 23 with severe toxaemia, of serum albumin concentration, distribution of albumin between plasma and extravascular compartments, exchange between the two compartments, and rate of albumin catabolism.

METHODS

At National Women's Hospital, Auckland, New Zealand blood volumes are estimated by a dilution technique based on radioiodinated serum albumin (RISA). The opportunity was thus available of investigating albumin dynamics by observing the decay of labelled plasma albumin activity after a blood volume measurement.

A number of blood samples were taken from each patient in the series in the week following isotope injection and a decay curve was constructed using the sample taken on completion of the blood volume determination as the initial activity. Allowance for radioactive decay was made by counting the samples at the same time on completion of each individual study. On the assumption of a two compartment model representing plasma and extravascular spaces a double exponential curve of the form

$$y = Ae^{-\alpha_1 t} + (1-A)e^{-\alpha_2 t} \qquad (1)$$

was fitted to the data by least squares analysis. In equation (1) y is plasma activity at time t, and A, α_1 and α_2 are parameters of the fitted curve. Serum albumin concentration was measured by the bromocresol green dye binding method.

Both two and three compartment models have been used in albumin turnover studies (Jacquez, 1972). The choice of a two compartment model in this study was based on the choice of the simplest model consistent with the data (Berman, 1963). The relevant programmes from the Harwell subroutine library have been used to implement a least squares technique of exponential-sum fitting (Hebden, 1973). The double exponential curve of equation (1) represents a considerable simplification over three or more exponentials as the optimal value of the coefficient A can be calculated directly from the optimal values of α_1 and α_2.

From the estimated parameters A, α_1 and α_2, figures for extravascular albumin mass, rate of exchange between plasma and extravascular compartments, and catabolism after allowing for any proteinuria were calculated as outlined by Matthews (1957). The number of points on the decay curve was kept to a minimum, usually five or six over a period of a week, sufficient for a double exponential curve. A greater number of samples, while desirable mathematically, was not considered to be justified on ethical grounds.

All subjects were in-patients of the hospital and all in the third trimester of singleton pregnancies except for one at 25 weeks and one at 26 weeks. Initially the 48 patients in the study were divided into a number of groups according to clinical diagnosis. However, it became apparent on analysing the results that there were only two statistically distinct groups: 23 patients with severe toxaemia and the remaining 25 patients. The definition of severe toxaemia was a diastolic blood pressure in the range 90 to 99 mm Hg with proteinuria greater than 1 g/day and oedema, or alternatively a diastolic blood pressure equal to 100 mm Hg or greater with the presence of at least one of the other two factors oedema and proteinuria.

RESULTS

Table I sets out the mean values for serum albumin concentration, plasma volume calculated from the initial blood volume determination, and the several results calculated from the RISA decay curves for the two groups of patients discussed. Plasma albumin mass expressed in g albumin/kg of maternal body weight was significantly lower for the severely toxaemic group (t-test, $p < 0 \cdot 001$), as would be expected from the low values of serum albumin concentration and plasma volume associated with this group. There was, however, no significant difference between the mean extravascular albumin masses of the two groups. Thus the

TABLE I

Mean values of serum albumin concentration, plasma volume and the variables calculated from the RISA decay curves for the 23 severely toxaemic and 25 other patients included in the study

	Severe toxaemia (n = 23)	Others (n = 25)	Significance of difference
Serum albumin concentration (g/l)	28·2	32·5	$p < 0 \cdot 001$
Plasma volume (l/kg)	0·0470	0·0549	$p < 0 \cdot 001$
Plasma albumin mass (g/kg)	1·31	1·78	$p < 0 \cdot 001$
Plasma albumin mass (g)	95	127	$p < 0 \cdot 001$
Extravascular albumin mass (g/kg)	1·11	1·24	NS
Extravascular albumin mass (g)	84	86	NS
Total extracellular albumin mass (g/kg)	2·42	3·02	$p < 0 \cdot 01$
Total extracellular albumin mass (g)	179	213	$p < 0 \cdot 05$
Extravascular/intravascular ratio	0·85	0·71	NS
Extravascular/total ratio	0·44	0·40	NS
Exchange (g/kg/hour)	0·041	0·052	NS
Exchange (per cent of total/hour)	1·75	1·80	NS
Catabolism (g/kg/day)	0·250	0·278	NS
Catabolism (per cent of total/day)	11·2	9·6	NS

NS = not significant

severely toxaemic patients had a lower total albumin mass than the other patients included in the study and not simply a different distribution between plasma and extravascular spaces ($p < 0{\cdot}001$).

The difference in intercompartmental exchange rate between the two groups which just fails to reach significance at the 5 per cent level appears to reflect the observed differences in total albumin. When expressed as a proportion of the total, averages of 1·75 per cent and 1·80 per cent respectively for the severely toxaemic patients and the others were exchanged hourly. Again, the absolute figures for catabolism when expressed as a proportion of total exchangeable albumin, were very similar with means of 11·2 per cent per day for the severely toxaemic patients and 9·6 per cent per day for the others.

DISCUSSION

The principal difference between the 23 severely toxaemic patients and the 25 others is the difference in plasma albumin mass. The lower total body albumin of the severely toxaemic patients, 2·42 g/kg, arises solely in the plasma compartment; the extravascular albumin masses are very similar both in absolute terms and on a body weight basis. The results for total extracellular albumin for both groups, 2·4 g/kg and 3·0 g/kg, are considerably less than the 4·0 g/kg typically quoted for non-pregnant women (Rothschild *et al*, 1972), although the difference is less marked when absolute values of total albumin are considered.

Direct comparison of the results of this study with those in the literature is difficult for a number of reasons. There are few studies of albumin metabolism in normal women since most reports describe investigations on male subjects (Sterling, 1951; Cohen *et al*, 1961; Beeken *et al*, 1962; Takeda and Reeve, 1963). In addition different methods of analysis yield different results from the same data and in particular the commonly used intercept method of estimating extravascular albumin does overestimate this space (Appendix). Finally the numbers of subjects included in many studies are small leading to fairly large standard errors in mean values. Table II lists the results of Steinfeld (1960) and Rossing (1967) for comparison with Table I.

TABLE II

Results in non-pregnant women

	Steinfeld (1960) 12 women	Rossing (1967) 19 women
Serum albumin (g/l)	37	43
Plasma volume (l/kg)	0·038	0·042
Plasma albumin (g/kg)	1·4	1·8
Extravascular albumin (g/kg)	2·5	2·2
EV/IV ratio	1·8	1·22
Total albumin (g/kg)	3·9	4·0
Catabolism (g/kg/day)	0·180	0·150

The result obtained in this study for total extracellular albumin of 213 g for the 'other' group is within 10 per cent of Rossing's figure of 229 g and within 15 per cent of Steinfeld's 250 g. Such differences could be explained in terms of differences in the methods of mathematical analysis alone. In addition all subjects in this study were hospital in-patients so even the 'others' grouping strictly cannot be regarded as normal. The results of this study thus suggest that total body albumin mass in absolute terms does not increase in pregnancy with increasing maternal weight so that total albumin in g/kg during the third trimester is lower than in non-pregnant women. There is however a redistribution to include a greater proportion in the plasma compartment. Such a redistribution would help maintain a reasonable plasma albumin concentration under conditions of an expanded plasma volume.

Most studies do not quote figures for exchange between plasma and extravascular compartments. Of the few that do so, Courtice *et al* (1951) quote a figure of 45 per cent of plasma protein leaked to the lymph per day. Results tabulated by Takeda and Reeve (1963) indicate about 100 per cent of plasma albumin or 42 per cent of the total is exchanged per day while Matthews (1957) also obtained a figure of 40 per cent in one human subject. Our results of 1·8 per cent of total albumin exchanged per hour for both groups of patients is very similar to published results for male subjects.

Thus the rate of capillary leakage of albumin does not appear to change in pregnancy.

Albumin catabolism however does seem to be increased substantially in pregnancy above the 0·150 to 0·180 g/kg/day of the non-pregnant state (Steinfeld, 1960; Rossing, 1967). After allowing for any proteinuria, the mean values for catabolism obtained in this study are 0·250 and 0·278 g/kg/day respectively for the severely toxaemic and 'other' groups, and the half lives of our experimental curves are correspondingly shorter than in the non-pregnant state. The difference probably reflects the increased metabolic demands of pregnancy. The assumption of a steady state over the course of each individual study implied in the mathematical analysis, that is a constant total extracellular albumin mass, implies that albumin synthesis must also be increased in pregnancy.

ACKNOWLEDGEMENTS

The author thanks the consultants of National Women's Hospital for permission to study their patients. The assistance and encouragement of Professor Sir William Liley throughout the project is gratefully acknowledged. I also thank Mr R. Coleman for collecting the blood samples and Dr P. McInerny for drawing my attention to the Harwell subroutine library. The study was supported by the Medical Research Council of New Zealand.

APPENDIX

CALCULATION OF THE RATIO OF EXTRAVASCULAR TO INTRAVASCULAR ALBUMIN MASS

Matthews (1957) states that the ratio of the size of the ith compartment to the size of compartment 1, the plasma compartment, is $\frac{K_{1i}}{K_{i1}}$. Using Matthew's notation, the ratio of extravascular (compartment 3) to intravascular albumin (compartment 1) is thus

$$EV/IV = \frac{K_{13}}{K_{31}} = \frac{C_1 C_2 (b_2 - b_1)^2}{(C_1 b_2 + C_2 b_1)^2} \tag{2}$$

where b_i, C_i ($i = 1, 2$) are the parameters of the plasma activity curve $X_1(t) = C_1 e^{-b_1 t} + C_2 e^{-b_2 t}$.

Dividing numerator and denominator of the right hand side of equation (2) by $C^2{}_1$,

$$EV/IV = \frac{C_2 (b_2 - b_1)^2}{C_1 (b_2 + \frac{C_2}{C_1} b_1)^2} \tag{3}$$

which is less than $\frac{C_2}{C_1}$ for all positive values of b_i and C_i ($i = 1, 2$).

The correct EV/IV ratio of equation (3) is thus less than that calculated by the intercept method, $\frac{C_2}{C_1}$, by an amount dependent on the parameters b_i, C_i ($i = 1, 2$).

EXAMPLE: Data of Sterling (1951), Figure 5. Sterling quotes half lives of 11·2 days and 0·6 days giving $b_1 = \cdot 0026/\text{hr}$ and $b_2 = \cdot 048/\text{hr}$. The intercept $C_1 = \frac{1160}{2760} = 0 \cdot 42$. As $C_2 = 1 - C_1 = 0 \cdot 58$ the plasma activity is therefore $X_1(t) = 0 \cdot 42 e^{-\cdot 0026 t} + 0 \cdot 58 e^{-\cdot 048 t}$ for a unit time of one hour.

From equation (3)

$$EV/IV = \frac{0 \cdot 58 (\cdot 048 - \cdot 0026)^2}{0 \cdot 42 (\cdot 048 + \frac{0 \cdot 58}{0 \cdot 42} \times \cdot 0026)^2} = 1 \cdot 07$$

compared with $\frac{0 \cdot 58}{0 \cdot 42} = 1 \cdot 38$ by the intercept method.

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THE POSSIBLE RELATION BETWEEN LATE PREGNANCY HYPERTENSION AND 5-HYDROXYTRYPTAMINE LEVELS IN MATERNAL BLOOD

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Summary

The concentrations of the vasoactive substance, 5-hydroxytryptamine (5–HT), were estimated in whole blood samples from 12 pregnant women with hypertension in the third trimester. These were compared with a control group of 15 normotensive pregnant women of the same gestation. The hypertensive patients were admitted for observation and the whole blood 5–HT estimated again after four days bed rest: in all patients the blood pressure had, by this time, returned to within the normal range. No significant difference was found between the mean blood 5–HT concentrations of the hypertensive and the normotensive groups. A small, but not statistically significant, rise occurred in the hypertensive group after four days bed rest. Thus this study gave no evidence that 5–HT is implicated in hypertension in pregnancy.

THE aetiology of hypertension developing in late pregnancy remains obscure. It has been suggested that biologically active amines play a role. Some workers have found increased levels of 5–HT in pre-eclampsia (Carter *et al*, 1962; Senior *et al*, 1963; Sotnikova, 1968; Ramadan *et al*, 1973; Grokhovskij, 1974) and some have not done so (Krupp and Krupp, 1960; Klinge *et al*, 1964). In previous work serum 5–HT concentrations have been measured by a biological assay (Krupp and Krupp, 1960; Senior *et al*, 1963; Klinge *et al*, 1964; Sotnikova, 1968) or by a fluorometric method (Ramadan *et al*, 1973). As the results are conflicting, we did a study of the possible association between hypertension in late pregnancy and 5–HT levels in whole blood measured by a sensitive fluorometric method.

PATIENTS AND METHODS

Two groups of pregnant women were studied. The first group (hypertensive patients) consisted of 9 primigravidae and 3 multigravidae (mean age 25, range 19 to 35) who had blood pressures in excess of 140 mm Hg systolic and 90 mm Hg diastolic at a routine visit to the antenatal clinic, having been normotensive previously. These patients were admitted for bed rest and observation. Medication was limited to sedation with 5 to 10 mg of oral nitrazepam at night. None of the patients had proteinuria or significant oedema. The second group (normotensive patients) consisted of 10 primigravidae and 5 multigravidae (mean age 23, range 18 to 33) who were attending an antenatal clinic.

The blood pressure was measured with the women in the supine position and high readings were checked after ten minutes rest. Urine was tested for protein by Albustix (Ames Company).

Blood collection

Blood samples were taken between 1400 and 1600 hours. The first sample was taken in the antenatal clinic. In the hypertensive patients, the second sample was taken in the ward after four days bed rest by which time the blood pressure had invariably returned to normal.

After application of a venous tourniquet, approximately 2·5 ml of blood were drawn from a cubital vein into a plastic disposable syringe. The blood sample was transferred immediately to a standard 2·5 ml lithium heparin plastic tube and mixed gently with the anticoagulant by repeated inversion of the tube. Blood samples were stored in the frozen state until the 5–HT estimations could be performed. It had been found in a previous study (Ashcroft *et al*, 1964) that whole blood 5–HT concentration remained unchanged for at least 10 days with this condition of storage.

Estimation of the 5–HT equivalent of venous blood

The method was a modification of that described by Ashcroft *et al* (1964) and gives an estimate of the concentration of 5-hydroxyindole compounds in whole blood. Under most conditions this represents the concentration of 5–HT. Only under exceptional circumstances, such as after 5-hydroxytrytophan administration, are other compounds likely to be present in concentrations which would contribute significantly to the estimates.

A 200 μl sample of the thawed mixed heparinized blood specimen was lysed in 550 μl deionized distilled water in a 1·5 ml plastic centrifuge tube. Protein was precipitated by 100 μl of 10 per cent (w/v) $ZnSO_47H_2O$ (BDH, Analar) followed by 50 μl M NaOH (BDH, Volumetric solution) with vortex mixing immediately after the addition of each reagent for 10 seconds and 20 seconds respectively. The concentration of 5-hydroxyindoles in 500 μl of the supernatant fluid, obtained by centrifugation at 1500 *g* for 4 minutes, was estimated by fluorimetry immediately after the addition of 200 μl of concentrated HCl (BDH: Aristar) containing ascorbic acid 0·5 mg/ml, the solution being prepared fresh for each batch of analyses. The fluorescence intensity of the acidified blood extract in a microcuvette was measured at 300 nm (activation) and 530 nm (emission), the wavelength characteristic of 5-hydroxyindoles, from the activation spectrum recorded from 200 nm to 380 nm. A Perkin Elmer MPF–2A spectrophotofluorometer was used with a secondary filter 39 (cut off at 390 nm), a 10 nm band pass for both monochromators, and at maximum sensitivity. The concentration of 5-hydroxyindoles in the blood sample was estimated by comparison with measurements obtained from aqueous standard solution of 5–HT processed along each batch of blood samples; the 'blank' fluorescence was allowed for by the method described by Ashcroft *et al* (1964). The estimates were corrected for the lower recovery of 5–HT added to blood, such recovery was assessed for each blood specimen by the concurrent analysis of a 200 μl sample to which 30 ng 5–HT had been added.

Duplicate estimates of both sample concentration and the recovery of added indole were obtained whenever possible. The mean recovery of 30 ng 5–HT added to blood samples was $61 \pm 10·75$ (SD) per cent ($n = 46$). Between duplicates of recovery estimates the mean difference, as a percentage of the 30 ng indole added, was $6·7 \pm 4·0$ (SD) ($n = 22$).

Comparison between the two groups was made using Student's 't' test. The significance of the difference in 5–HT concentration before and after bed rest in hypertensive patients was determined from a paired 't' test.

RESULTS

Blood pressure

The mean systolic blood pressures of the hypertensive and normotensive patients at the

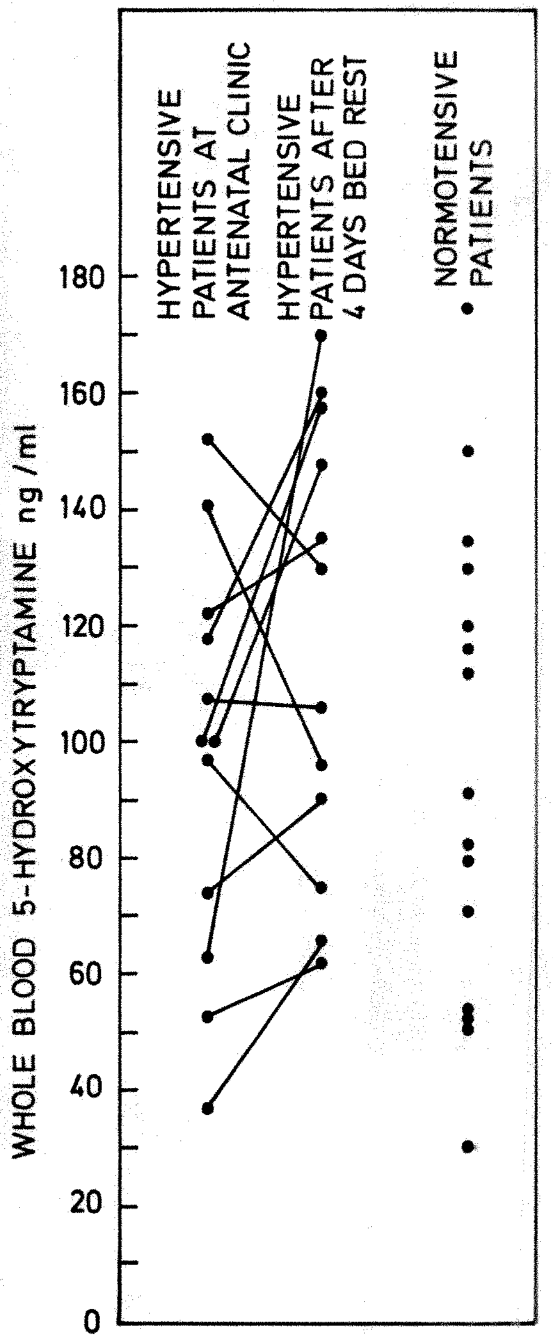

FIG. 1

Distribution of whole blood 5-hydroxytryptamine concentrations in hypertensive patients before and after four days bed rest and in normotensive patients. All patients were in the third trimester.

antenatal clinic were respectively 152±12 (SD) mm Hg and 129±10 mm Hg while the respective mean diastolic pressures were 101±7 mm Hg and 80±5 mm Hg. After four days bed rest, the mean systolic and diastolic pressures in hypertensive patients fell to 132±6 mm Hg and 83±5 mm Hg respectively.

Blood 5–HT

The mean whole blood 5–HT concentration in hypertensive patients was 98±35 (SD) ng/ml and in normotensive patients it was 95±41 ng/ml. Thus the concentrations did not differ significantly between the two groups. After four days bed rest the whole blood 5–HT concentration in hypertensive patients was 116±39 (SD) ng/ml. A paired 't' test showed no significant difference ($0\cdot1 < P < 0\cdot2$) between estimates in samples obtained before and after bed rest. The detailed results are plotted in Figure 1.

DISCUSSION

Much interest has centred on the possible role of 5–HT in the aetiology of pre-eclampsia. Various workers using either biological or fluorometric methods have measured 5–HT in serum and placentae in normal patients and patients with pre-eclampsia with contradictory results.

Krupp and Krupp (1960), using a biological assay, found higher plasma 5–HT levels in pre-eclampsia. Their study, however, included a number of patients with progressive pre-eclampsia in whom no 5–HT could be detected. Klinge *et al* (1964) estimated biologically the 5–HT content of specimens of human placentae and uterus obtained at Caesarean section from normal patients and patients with pre-eclampsia and found no differences. Sotnikova (1968) using biological assay found increased blood 5–HT concentrations in late pre-eclampsia. Grokhovskij (1974) reported decreased levels of 5–hydroxyindole acetic acid in the urine of patients with pre-eclampsia and stated, without any supporting evidence, that this may be associated with increased circulating 5–HT levels. Senior *et al* (1963) also employing a biological assay found increased levels of 5–HT in placentae of patients with pre-eclampsia but were unable to detect any difference in maternal blood levels. Ramadan *et al* (1973) reported increased levels of serum 5–HT measured fluorometrically in pre-eclampsia. The authors also found an increase in the 5–HT content of placentae from patients with pre-eclampsia. However, they failed to find any relation between serum 5–HT concentration and the level of blood pressure.

The variations in methodology may account for the conflict in opinion about the role of 5–HT in pregnancy hypertension. In whole blood, 5–HT is mostly confined to the platelets, much smaller amounts being present free in plasma. Serum 5–HT estimations bear little relation to free plasma 5–HT because of variable amounts of 5–HT released from platelets during clot formation (Gowenlock and Platt, 1963). We therefore measured the whole blood 5–HT concentration.

We have not been able to demonstrate that hypertension in pregnancy is associated with raised whole blood 5–HT concentrations. However, only the free circulating amine may be important and changes in the concentration in this fraction may be too small to be reflected by a significant difference between the means of the whole blood 5–HT concentrations of the normotensive and hypertensive patients.

Both the normotensive and hypertensive patients showed large differences in the 5–HT concentrations between individuals. This marked individual variation has been noted previously by Ashcroft *et al* (1964) who did, however, find that the value for the same individual tended to remain fairly constant from day to day with no tendency to diurnal or post-prandial variations. Thus the comparison of 5–HT concentrations before and after bed rest for hypertension should have more significance than the comparison between the normotensive and hypertensive patients. But bed rest produced no consistent or statistically significant changes in the whole blood 5–HT levels of hypertensive patients (Fig. 1). This speaks against the role for 5–HT in the aetiology of pregnancy hypertension. But further investigations such as parallel studies of blood and urine 5–HT levels are necessary before a definite conclusion can be reached. This is because work with animals has shown renal clearance of 5–HT is almost complete (Sanner and Wortman, 1962; Weiner and Mudge, 1964) and thus the urinary 5–HT level may be a better indicator of the amount of free circulating amine. Such studies are in hand.

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OSCILLATION-FREQUENCY AND BASELINE FETAL HEART RATE IN THE LAST 30 MINUTES OF LABOUR

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Summary

The last 120 minutes of 394 intrapartum fetal heart rate (FHR) records of high technical quality were evaluated. Baseline FHR was estimated and oscillation frequency (OF) counted for each minute. There was a highly significant association between the baseline FHR and the number of oscillations/minute; an increase in baseline FHR of 10 beats/minute was associated with a fall of about one oscillation/minute. The highest mean OF ($9\cdot6 \pm 4\cdot8$ events/minute) was found in fetuses with an umbilical artery pH of between 7·20 and 7·25. The baseline FHR should be taken into account when assessing fetal condition.

FETAL heart rate (FHR) variability seems to be an important index of fetal health (Hammacher *et al*, 1968; Beard *et al*, 1971; Yeh *et al*, 1973; Boehm *et al*, 1977). Normal variability reflects fetal well-being whereas lack of baseline fluctuation may be associated with fetal compromise. Recently we demonstrated that the oscillation frequency (OF) of the baseline FHR is closely associated with the oscillation amplitude (OA) per minute; reduction of OA is followed normally by a decrease in the OF (Roemer and Heinzl, 1977). In this context, OF refers to the number of visually detectable peaks in baseline FHR, that is the number of turning-points from frequency acceleration to deceleration and *vice versa* (Fig. 1). While this descriptive approach reflects our clinical impression of FHR variability, it is not equal to true baseline fluctuation which cannot be evaluated accurately without computers (De Haan *et al*, 1971; Yeh *et al*, 1973; Laros *et al*, 1977; Grothe *et al*, 1977).

Clinical experience seems to indicate that in vigorous infants OF is not only associated with OA (Roemer and Heinzl, 1977; Fischer, 1976) but is influenced also by the level of the baseline. This observation, already established by other authors (De Haan *et al*, 1971 and 1972) shoulp be of some clinical importance when the diagnosis of fetal distress is assessed from baseline FHR criteria. The following study was designed to test this hypothesis.

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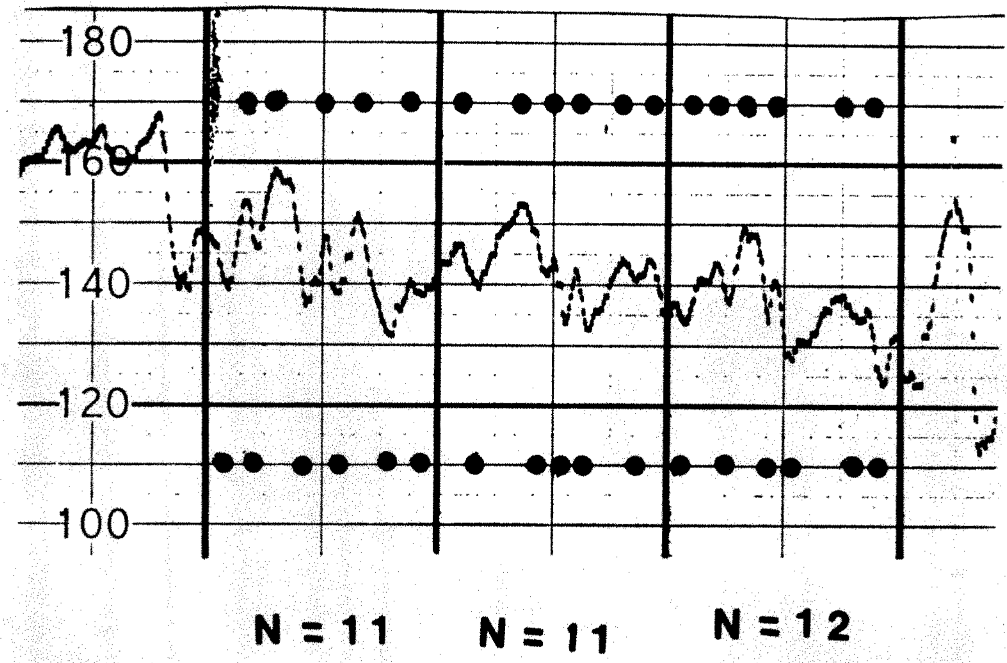

FIG. 1

Method of calculating the oscillation frequency (OF) of basal FHR: all visually detectable turning points of each minute of the baseline were counted (N = 11 and 12/minute). The oscillation amplitude (OA) was measured in mm using a specially made caliper and then converted into beats/minute.

METHODS

The last 120 minutes of 396 intrapartum FHR tracings (47 520 minutes) were divided in 12 segments each of 10 minutes duration and visually analysed with simple tools like compasses and calipers. Four variables were recorded: (1) the average baseline fetal heart rate (FHR) in beats/minute; (2) the oscillation amplitude per minute (OA), that is the distance from the highest to the lowest point in baseline FHR and measured for convenience in mm by a caliper designed by Roemer and Heinzl (1977) and transformed into beats/minute by the computer; (3) the oscillation frequency; and (4) important details of each deceleration and acceleration as described previously (Roemer and Heinzl, 1977). All variables were coded together with clinical and biochemical findings onto 25 punched cards and analysed by computer. All FHR records were obtained by direct techniques where the R-wave of the FECG is used as the trigger, together with beat-to-beat processing of the electronic signal. The paper speed was 2 cm/minute. FHR tracings which were interrupted by Caesarean section, or difficult vaginal deliveries, were excluded. The pH in blood from the umbilical artery (UA) and vein (UV) was measured with three different pH measuring systems (Radiometer ABL II, BMS III, AME I) in all but two patients. In the present study the analysis of only the 30 minutes immediately preceding delivery is presented, in order to focus the association between the biochemical outcome of the fetus and baseline criteria of FHR. The so-called normal frequency range (Fischer, 1976) between 120 and 160 beats/minute was divided into four 10 beats/minute zones (Table I). The empirical distribution of the pH-values in the UA was divided into six sample groups (Fig. 2). All distributions of variables were compared with the non-parametric Kolmogorov-Smirnof two sample test (Sigel, 1956).

RESULTS

Figure 3 shows five frequency distributions of the OF at different levels of baseline FHR in 394 fetuses. The mean pH was $7\cdot240 \pm 0\cdot086$. All

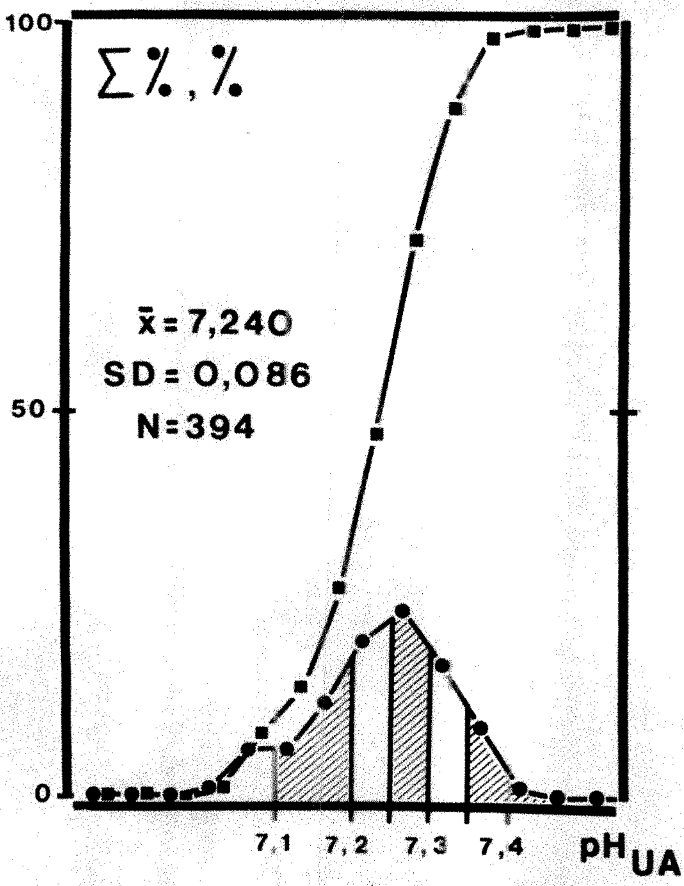

FIG. 2

Empirical frequency distribution of 394 pH values measured in umbilical arterial (UA) blood. Median = 7·250, pH <7·200 = 27·1 per cent. The hatched and clear areas indicate the six pH-sample groups chosen for further analysis.

five empirical OF distributions differed from each other at a high level of significance. These data prove again (Roemer and Heinzl, 1977) that the OF is a function of the baseline level during the last 30 minutes of labour (Table I). To elucidate these phenomena further, subsamples of fetuses with a similar umbilical artery pH were compared (Tables II and III). From these data (Table II) it is evident that fetuses with an umbilical artery pH between 7·201 and 7·250 (Mean ± SD = 7·227 ± 0·020) had the highest mean OF of 9·6 ± 4·8.

A lower umbilical artery pH was associated with a reduction of OF which was highly significant (α < 0·001) only below a pH of 7·100. Increase of pH values was associated with a continuous but small and not significant decrease of OF up to 8·9 ± 4·3 in fetuses with pH values above 7·350. The mean OA was increased to 23·6 ± 15·4 beats/minute in fetuses with an umbilical artery pH of 7·101 to 7·200. Further decrease of the pH did not alter OA significantly whereas OF was clearly and significantly decreased. Mean baseline FHR showed a significant tendency to higher values in fetuses with preacidotic pH values; but the use of mean values conceals the increased incidence of mild and severe bradycardia and tachycardia associated with reduced umbilical artery pH (Table II). For example the incidence of a baseline FHR below 100 beats/minute steadily increased from 3·6 per cent to 11·8 per cent

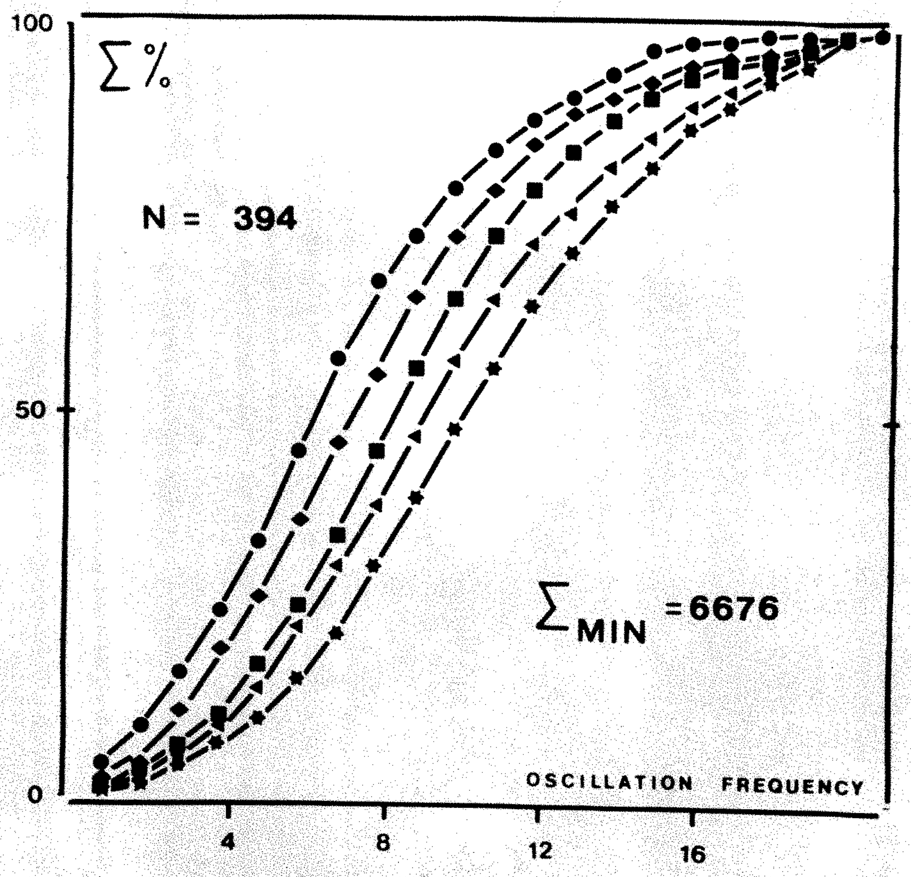

FIG. 3

Cumulative frequency distribution of oscillation frequency/minute (OF) in five basal FHR groups in 394 fetuses during the last 30 minutes of labour. ● = >160 bpm; ◆ = 151–160 bpm; ■ = 141–150 bpm; ▲ = 131–140 bpm; * = 130 or less bpm. The five distributions differ from each other with a high level of significance ($p < 0{\cdot}01$, (Table I)).

TABLE I

The oscillation frequency per minute related to baseline fetal heart rate (FHR)

Baseline FHR (beats/minute)	Duration of observation (minutes)	Oscillation frequency/minute		Centile			Kolmo II	
		Mean	SD	5th	50th	95th	Z	α
>160	468	6·3	3·9	1	6	14		
160–151	567	7·3	4·2	2	7	15	1·791	0·0032
150–141	1082	8·3	4·2	2	8	16	2·263	0·000071
140–131	1544	9·2	4·7	2	9	18	2·146	0·00019
130–121	1474	10·0	4·6	3	10	18	2·556	0·000004
≦120	1541	10·2	4·5	3	10	18	0·984	0·286

Total number of observations = 394.
Total observation time = 6676 minutes.
Umbilical artery pH (Mean ± SD) = 7·240 ± 0·086.

TABLE II

The oscillation frequency, oscillation amplitude and baseline fetal heart rate (FHR) related to the pH of umbilical artery blood. Number of minutes of observation in parenthesis

	Umbilical artery pH					
	≦7·100 (n = 35)	7·101–7·200 (n = 86)	7·201–7·250 (n = 77)	7·251–7·300 (n = 111)	7·301–7·350 (n = 53)	>7·350 (n = 32)
Oscillation frequency/minute	7·3 ± 4·3*** (497)	9·1 ± 4·5 (1313)	9·6 ± 4·8 (1373)	9·3 ± 4·7 (1868)	9·2 ± 4·4 (972)	8·9 ± 4·3 (653)
Oscillation amplitude (beats/minute)	22·0 ± 15·3 (496)	23·6 ± 15·4** (1313)	21·7 ± 13·7* (1373)	21·9 ± 14·0** (1868)	20·7 ± 13·4 (972)	20·6 ± 11·6 (653)
Baseline FHR (beats/minute)	131 ± 27*** (803)	134 ± 22*** (2059)	133 ± 20* (1902)	133 ± 18 (2772)	130 ± 17 (1369)	131 ± 17*** (856)
< 100 bpm (%)	11·8	7·5	6·6	4·3	3·8	3·6
< 80 bpm (%)	6·2	2·0	1·2	0·9	1·0	1·1
> 160 bpm (%)	14·0	13·4	8·6	6·5	3·4	5·2
> 180 bpm (%)	2·2	1·1	0·2	0·4	0·1	0

*: α <0·05, **: α <0·01, ***: α <0·001 (Kolmogorov-Smirnof).

TABLE III

The relation of oscillation frequency to baseline fetal heart rate (FHR) in six groups divided according to the pH of umbilical artery blood. Number of minutes of observation in parenthesis

Baseline FHR (beats/minute)	Oscillation frequency/minute (Mean ± SD)					
	Umbilical artery pH					
	≦7·100	7·101–7·200	7·201–7·250	7·251–7·300	7·301–7·350	>7·350
> 160	4·2 ± 2·7 (67)	6·5 ± 4·2 (153)	7·7 ± 3·8 (111)	6·2 ± 3·6 (90)	5·3 ± 2·6 (29)	6·9 ± 4·7 (18)
160–151	5·3 ± 3·7 (50)	7·7* ± 4·2 (132)	7·8 ± 4·9 (127)	7·7 ± 4·2 (161)	7·0 ± 2·6 (63)	6·2 ± 4·0 (34)
150–141	6·9 ± 3·7 (82)	8·6 ± 4·2 (183)	8·9 ± 4·3 (222)	8·2 ± 4·5 (314)	8·4 ± 3·7 (175)	7·9 ± 3·6 (106)
140–131	8·1 ± 3·9 (75)	9·4 ± 4·7 (258)	9·7 ± 5·0 (314)	9·3 ± 4·8 (486)	9·2 ± 4·6 (254)	8·0 ± 4·4 (157)
130–121	9·8* ± 3·5 (71)	9·7 ± 4·6 (276)	10·6 ± 4·8 (317)	10·2 ± 4·7 (404)	10·2 ± 5·0 (194)	9·3 ± 3·9 (212)
≦120	8·1** ± 4·7 (152)	10·5* ± 4·1 (309)	10·2 ± 4·8 (287)	10·6 ± 4·6 (410)	10·6 ± 4·1 (257)	11·0 ± 4·5 (126)
Umbilical artery pH	7·064 ± 0·030 (n = 35)	7·168 ± 0·037 (n = 86)	7·227 ± 0·022 (n = 77)	7·278 ± 0·041 (n = 111)	7·328 ± 0·031 (n = 53)	7·378 ± 0·016 (n = 32)

* α <0·05, ** α <0·01, (Kolmogorov-Smirnof)
Total number of observations = 394.
Umbilical artery pH (Mean ± SD) = 7·240 ± 0·086.

with decreasing pH, and the incidence of bradycardia below 80 beats/minute during the last 30 minutes of parturition rose from about 1 per cent in fetuses with a normal umbilical artery pH to 6 per cent when the pH was 7·10 or below. A similar numerical pattern was encountered in mild and severe tachycardia. One may conclude therefore that the higher the percentage of severe bradycardia and tachycardia the higher is the chance of acidosis. It is interesting to note, however, that in acidotic fetuses severe bradycardia was encountered almost three times more frequently than severe tachycardia. From the clinical point of view, the mean baseline frequency was nearly constant throughout the six pH sample groups; only the variation about the mean was remarkably increased in association with fetal acidosis.

The relation of OF to baseline FHR in each of the six pH groups is shown in Table III. Two major observations can be made. Firstly, in all pH sample groups the OF decreased with increasing mean baseline FHR. This clear-cut phenomenon is somewhat concealed in the overall figures by the second observation which is that with decreasing umbilical artery pH there is first a distinct increase of OF in fetuses with mild acidosis (pH 7·201 to 7·250) and then a significant decrease of OF in fetuses with severe acidosis (umbilical artery pH 7·100 or less). Since the mean of a sample is not always the most informative statistic, the median and centiles of each distribution were computed; these data are given in Tables IV and V. It is evident that mild acidosis leads to higher proportions of time with rapid fluctuations, that is with higher OF. Moreover it is clear that OF alone, or even in combination with the baseline

TABLE IV

The relation of median oscillation frequency to baseline fetal heart rate (FHR) in six groups divided according to the pH of umbilical artery blood

Baseline FHR (beats/minute)	Oscillation frequency/minute (median)					
	Umbilical artery pH					
	<7·100 (n = 36)	7·101–7·200 (n = 86)	7·201–7·250 (n = 77)	7·251–7·300 (n = 111)	7·301–7·350 (n = 53)	>7·350 (n = 32)
>160	4**	6*	7*	6	5	6
160–151	5**	7·5	7	8	7	5·5
150–141	6**	8	8	8	8	8
140–131	8	9	9	9	9*	8
130–121	9	9	10	10	10*	9
≦120	8***	11*	9*	10	10	11
Duration of observation (minutes)	497	1311	1378	1865	972	653

*: α <0·05, **: α <0·01, ***: α <0·001 (Kolmogorov-Smirnof).
Total number of observations = 394.
Total observation time = 6676 minutes.

TABLE V

Oscillation frequency centiles in six groups divided according to the pH of umbilical artery blood

Centile	Oscillation frequency/minute					
	Umbilical artery pH					
	≦7·100	7·101–7·200	7·201–7·250	7·251–7·300	7·301–7·350	>7·350
5th	0	2	2·5	2	3	2
10th	2	3	4	3	4	3
25th	4	6	6	6	6	6
50th	7	9	9	9	9	9
75th	10	12	13	13	12	11·5
90th	13	15	16	16	15	14
95th	15	17	19	18	17	17
99th	18	22	22	21	20	19

FHR, is a poor measure of fetal distress. If OF is markedly reduced, severe fetal acidosis should be anticipated, assuming that no other factor than asphyxia is involved; these would include immaturity, fetal inactivity or 'quiet sleep' (Dalton *et al*, 1977) as well as maternal drug administration (Dalton *et al*, 1976; Babaknia and Niebyl, 1978; De Haan *et al*, 1972).

DISCUSSION

The computer is a useful tool for analysis of large amounts of data, but erroneous data cause serious problems of analysis and the input of FHR is usually contaminated with false signals throughout delivery. We decided therefore to evaluate FHR patterns by visual analysis of records of high technical quality, although it should be emphasized that this time consuming and troublesome method of analysis is only a semi-quantitative compromise. It has the advantage of being closely related to clinical observations and therefore may be of some help to the obstetrician. It is well known that OF is greatly influenced by maternal drug administration (Hammacher *et al*, 1968; Boehm and Growdon, 1974; Fischer, 1976; Babaknia and Niebyl, 1978) and a variety of other factors which were not analyzed in this study. Although drugs (mainly given during the first stage of labour), cord entanglement and other clinical circumstances were documented, we decided to disregard these factors in this first study. Clinical studies dealing with these factors are in preparation. Similar results have been described already by De Haan *et al* (1971 and 1972) using the short-term irregularity index (S.T.I.-Index). De Haan *et al* (1971 and 1972) observed an almost complete disappearance of the short-term irregularity around a frequency of 150 to 160 beats/minute. They concluded "that the regulation system of the fetal heart action is saturated to one site of the system and the frequency by which the disappearance of the short-term irregularity is almost complete can be called tachycardia". Our results seem to be in agreement with these observations although the two measures involved, short-term irregularity index and OF, are barely comparable.

It is not fully understood why beat-to-beat variability, which is statistically closely associated with OF, becomes reduced with increasing baseline FHR (Fig. 3). A possible explanation might be that with increasing baseline FHR the myocardial ATP-consuming processes and the requirement for ATP are increased. Sufficient amounts of energy-binding compounds can only be provided if there is enough time between individual contractions. Time is also necessary for regeneration of the membrane potentials. This can be achieved only if the time intervals between the energy-consuming periods (systole) become more equal, that is if beat-to-beat variability is significantly reduced. In the case of hypoxia the synthesis of energy-binding compounds is depressed and consequently these regulatory principles should become more evident: the lack of beat-to-beat fluctuation in acidotic fetuses is obviously more pronounced in tachycardia than in mild bradycardia.

There seemed to be a tendency for OF to increase in some fetuses with a mild acidosis since the proportion of time in baseline FHR with rapid fluctuations was greatest in this group. We have no plausible explanation for this observation. It seems possible that this is a sign of early fetal distress (Dalton *et al*, 1977; Miller *et al*, 1978), but it might also be a reaction of the central nervous regulation centres to such stimuli as hypercapnia (Dalton *et al*, 1976).

Regardless of the physiological background of these phenomena, they are clinically valuable and may help the obstetrician in the management of high risk patients during labour.

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DISCREPANCIES IN THE HISTOLOGICAL DIAGNOSIS OF HYDATIDIFORM MOLE

BY

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Summary

Sections of tissue from 256 patients on which a histological diagnosis of hydatidiform mole had been made were reviewed by a visiting pathologist. There was agreement between the reporting pathologists and the visiting pathologist in only 55·1 per cent of cases, a less severe diagnosis was made by the visiting pathologist in 42·2 per cent of cases. The disagreement was as high as 66·6 per cent with pathologists who rarely worked in the gynaecological field. This study casts serious doubt on the consistency of the histological diagnosis of hydatidiform mole made in any one laboratory and between different laboratories. It is suggested that in epidemiological studies the diagnosis should be made by a panel of pathologists using strict histological criteria and in an individual laboratory diagnosis should be made or confirmed by a pathologist experienced in gynaecological pathology.

THE histological diagnosis of hydatidiform mole is usually regarded as easy and is made without much difficulty but occasionally the appearances may be confused with other abnormalities of the early conceptus. This is especially true if strict criteria for the diagnosis of hydatidiform mole are not used. Errors in diagnosis may lead to disastrous consequences if an invasive mole or choriocarcinoma is missed, to excessive and ill-directed treatment if a bland abnormality of the conceptus is not recognised, or to wrong estimates of the prevalence of the disease.

To assess whether or not the diagnosis of hydatidiform mole presents any difficulty and, if so, to determine its significance, we compared the histological diagnoses of hydatidiform mole made by a visiting pathologist from Manchester University UK (F.A.L.) with those of the reporting pathologists in The University of Shiraz, Iran.

MATERIAL AND METHODS

In this study, tissue sections of 256 patients diagnosed as suffering from hydatidiform mole by the pathologists of the Shiraz Medical Institutions for the years 1969 to 1974 were reviewed by the visiting pathologist (F.A.L.).

Only the routine sections, stained by haematoxylin and eosin used for the initial pretreatment diagnosis, were reviewed. Neither the naked eye appearance of the tissue nor the clinical aspects of the disease were considered in this re-evaluation. Cases of overt or 'borderline' choriocarcinoma were excluded.

The visiting pathologist used the following definitions based on the studies of Hertig and Mansell (1956) and Elston (1976) which he considered consistent with the descriptions given in recent reviews (Bigelow, 1977; Fox, 1978).

Syncytial endometritis (or placental site reaction). In this condition the placental bed is infiltrated with wandering 'trophoblastic cells' in the absence of villi.

Retained products of conception. Here the villi, apart from ischaemic degeneration and necrosis, are usually normal but they may show (i) apparent lack of blood vessels because these have closed down following death of the fetus and (ii) slight oedema.

Abnormal conceptus (or hydropic abortion). This is diagnosed if a moderate number of villi are enlarged and oedematous with or without visible blood vessels but with no trophoblastic hyperplasia.

Borderline hydatidiform mole. This shows features similar to an abnormal conceptus but with mild trophoblastic hyperplasia.

Hydatidiform mole. This shows three characteristic features: (i) enlargement and hydropic degeneration of villi, (ii) the absence of visible blood vessels in the villi and (iii) trophoblastic hyperplasia.

Borderline invasive mole. This term is used when the appearances are suggestive but not conclusive of invasion (the inadequate specimen is excluded).

Invasive mole. This is diagnosed when there is clear evidence of the villi of a hydatidiform mole invading the myometrium or its blood vessels.

A questionnaire incorporating the above definitions was sent to ten Shiraz pathologists and they were asked whether they agreed or disagreed with them.

RESULTS

Table I shows that there was considerable disagreement between the Iranian pathologists and the visiting pathologist particularly with respect to the definitions of abnormal conceptus and borderline invasive mole and to a less extent with the definition of invasive mole. The Shiraz pathologists also differed among themselves in the use of these terms.

TABLE I

Histological definitions given by a visting pathologist (F.A.L.) and the opinion of ten Shiraz pathologists

	Opinion of Shiraz pathologists	
F.A.L.'s definition	Agree	Disagree
Syncytial endometritis	10	0
Products of normal conception	8	2
Products of abnormal conception	4	6
Borderline hydatidiform mole	10	0
Hydatidiform mole	8	2
Possibly invasive mole	4	6
Invasive mole	6	4

In 185 cases it was possible to identify the pathologist in Shiraz who had made the original diagnosis. These were used for further analysis (Table II). Most of the sections had been initially examined by one of four pathologists A, B, C or D; the remainder had been examined by a number of different pathologists, each of whom had seen only a few cases. F.A.L. agreed with the reporting pathologist in only 55·1 per cent of cases and in 42·2 per cent F.A.L. apparently underdiagnosed the severity of the lesion whilst in 2·7 per cent he over diagnosed the severity. The discrepancies in diagnosis were not uniform. Thus Table II shows that the differences between pathologist A and F.A.L. were only 9·6 per cent and between D and F.A.L. 17·8 per cent, whereas the differences between F.A.L. and B and F.A.L. and C were much larger, being 64 and 46·3 per cent respectively. Moreover, the seven 'other' less experienced in gynaecological pathology showed the greatest variation, namely 66·6 per cent.

In all, there were 256 specimens which F.A.L. had an opportunity to re-examine. He agreed with a diagnosis of hydatidiform mole in 141 out of 253 instances; the remainder he reclassified as syncytial endometritis (4 specimens), products of normal conception (39 specimens), products of abnormal conception (47 specimens), borderline

TABLE II

Discrepancies between Shiraz pathologists histological diagnoses of hydatidiform mole and those of a vistiing pathologist. (*185 cases*)

	Agree		F.A.L.'s diagnosis less serious		F.A.L.'s diagnosis more serious		Total No. of discrepancies	
Pathologist	No.	per cent	No.	per cent	No.	per cent	No.	per cent
A	28	90·3	2	6·5	1	3·2	3	9·6
B	12	35·3	21	61·8	1	2·9	22	64
C	22	53·7	19	46·3	0	0	19	46·3
D	23	82·1	4	14·3	1	3·6	5	17·8
Others	17	33·3	32	62·7	2	3·9	34	66·6
Total	102	55·1	78	42·2	5	2·7	83	44·9

hydatidiform mole (16 specimens), possibly invasive mole (4 specimens) and invasive mole (2 specimens). F.A.L. and the Shiraz pathologists agreed about two specimens showing syncytial endometritis, and one labelled as invasive mole by the Iranians was reclassified as a 'possibly invasive mole' by F.A.L.

DISCUSSION

It is not unusual to find that a second look at a series of similar lesions by a pathologist, who is not responsible to the clinicians in charge, results in somewhat less severe diagnoses than those originally made (Cocker *et al*, 1968). Presumably this is because the pathologist now knows that the treatment is no longer dependent on his opinion and he can make a 'scientific' rather than a 'clinically safe' judgement. Thus, it is not surprising that in this series there were differences between the visiting and reporting pathologists, although the extent of these discrepancies is unexpected.

If it is assumed that the different types of trophoblastic lesion were randomly distributed between the histological sections examined by the pathologists in the Shiraz Medical School, the difference between the diagnoses of each individual pathologist and the visiting pathologist would be expected to be approximately the same. However, there were considerable differences between the individual pathologists in the Shiraz Medical School which suggests they were not using the same diagnostic criteria as each other. This parallels the differences recorded in Table I but it does not imply that any one pathologist (including F.A.L.) is more correct than another. Wilson and Burke (1957, 1959 and 1960) have shown similar discrepancies between pathologists when diagnosing atypical lesions of the bronchus and similar differences between pathologists have been shown to occur in the diagnosis of Hodgkin's disease (Coppleson *et al*, 1970).

Whilst bearing the above statement in mind it seems likely that pathologists B and C included a number of cases of abnormal conceptus in their diagnoses of hydatidiform mole. The pathology of abortion and the abnormal conceptus has recently been reviewed by a number of authorities on placental pathology (Phillipe, 1974; Bigelow, 1977; Fox, 1978). In 20 to 40 per cent of placentas from abortuses, the villi are swollen and oedematous and either hypovascular or avascular. Moreover, in recent years it has become apparent that chromosomal abnormality is one of the most important factors in spontaneous abortion, the incidence given by different workers varying from 24 to 55 per cent (Fox, 1978). Many of these chromosomal abnormalities may be accompanied by characteristic naked eye or histological changes. Rushton (1977, personal communication) has suggested that attention to such details and correlation with the stage of gestation might serve to distinguish some abnormal conceptuses from hydatidiform moles; in particular the early cases are more likely to be abnormal conceptuses whilst the later are much more probably hydatidiform moles. Attention to such morphological and clinical details were not used by the visiting pathologist.

If such differences in histological diagnosis apply in a wider context, say in the Far East, Europe and America, the overall pattern of the epidemiology of hydatidiform mole may be distorted. This may not invalidate the general conclusions that the disease is far more common in the East than in the West but it may obscure the search for aetiological factors such as the relation of environmental, genetic and chromosomal factors. Javey and Sajadi (1978) have discussed the variations in the prevalence of hydatidiform mole in Iran in relation to nutritional standards and dietary habits but, as shown here, part of the apparent variation may be due to differences in diagnostic criteria. Further, if in a therapeutic trial patients with abnormal conceptuses are included with those having hydatidiform moles the clinical significance of the test will be biased. Whilst for an individual patient under- or over-diagnosis of hydatidiform mole may be fraught with danger since it may lead to inappropriate management.

This study serves to emphasize the need for strict histological criteria for the diagnosis of hydatidiform mole and for the products of normal and abnormal conception. In an epidemiological study a panel of pathologists might well review the histological diagnoses and in a hospital laboratory the histological diagnosis of individual cases should be confirmed by a pathologist experienced in gynaecological pathology.

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PRIMARY DYSMENORRHOEA AND VASOPRESSIN

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Summary

The circulating concentrations of arginine vasopressin on day 1 and on day 5 to 7 of the menstrual cycle were measured by radioimmunoassay in six women with primary dysmenorrhoea and five controls. All women had ovulated in the previous cycle, as indicated by the mid-luteal phase plasma progesterone levels and had normal plasma osmolality and sodium concentrations. The plasma concentrations of progesterone and oestradiol on day 1 of the cycle were also measured, and no significant difference between the two groups was seen. In women with dysmenorrhoea the vasopressin concentration on day 1 was $0 \cdot 40 \pm 0 \cdot 038$ (SE) μU/ml which was significantly higher than the concentration in normal women on the same day of the cycle $0 \cdot 20 \pm 0 \cdot 063$ (SE) μU/ml ($p < 0 \cdot 01$). The plasma concentration of vasopressin in normal women on day 5 to 7 was $0 \cdot 68 \pm 0 \cdot 119$ (SE) μU/ml which was significantly higher than on day 1 ($p < 0 \cdot 01$). A significant difference between day 1 and day 5 to 7 was not seen in dysmenorrheic women, possibly because the values were already elevated on day 1. The results suggest that vasopressin could be a factor of aetiological importance in primary dysmenorrhoea, and also indicate that the vasopressin concentration in plasma can vary according to the day of the menstrual cycle.

IN primary dysmenorrhoea the pain seems to be associated with increased myometrial activity (Woodbury *et al*, 1947; Filler and Hall, 1970; Åkerlund *et al*, 1976; Csapo *et al*, 1977) and decreased blood flow in the uterus (Åkerlund *et al*, 1976). Vasopressin could be of aetiological importance in this condition, since administration of vasopressin to non-pregnant women at the onset of menstruation causes a marked increase in uterine activity (Joelsson *et al*, 1966; Coutinho and Lopes, 1968; Bengtsson, 1970; Åkerlund and Andersson, 1976) and a decrease in uterine blood flow (Åkerlund and Andersson, 1976). Furthermore, recordings of uterine blood flow from women with dysmenorrhoea often show cyclic variations with a periodicity of 15 to 30 minutes (Åkerlund *et al*, 1976), which is suggestive of vasopressin involvement, since, like other hypothalamic hormones, it may be secreted episodically (Weitzman *et al*, 1977). The concentration of vasopressin in plasma can now be accurately determined by radioimmunoassay (Chard and Forsling, 1976) and this allowed a study of the possible role of vasopressin in primary dysmenorrhoea.

METHODS

Subjects

Ten volunteers suffering from severe primary dysmenorrhoea and ten gynaecologically healthy women with no menstrual pains participated in the study. The women with dysmenorrhoea reported symptoms occurring two to four years after their menarche and at the time of the study they were so disabled by the condition that they were unable to work for one to three days during each menstruation. Women from neither group had signs of pelvic or gynaecological disease. None of the women used hormonal contraception or had an intrauterine contraceptive device. No medication was taken during the period of the study.

Procedure

All women had regular cycles with an interval of 26 to 33 days between periods which lasted between four and seven days. Venous blood samples were obtained on the first and on the fifth to seventh day of the cycle. On the morning of sampling the women had a normal breakfast and did not smoke. The first sample (I) was obtained around 0900 hours, after 30 minutes rest in sitting position. A second sample (II) was obtained 30 minutes later. At the time of sampling the women with dysmenorrhoea were asked to report on the severity of their symptoms. The sampling procedure was repeated on the fifth to seventh days of the cycle (III and IV). For vasopressin analysis, 10 ml of blood were obtained in chilled plastic tubes. They were immediately centrifuged at 2500 *g* for 25 minutes at 5°C; the plasma was separated, frozen and kept at −20°C. Plasma osmolality and sodium concentration were measured in samples obtained immediately after the vasopressin samples. In addition, the concentrations of oestradiol and progesterone were measured by radioimmunoassay in plasma taken on the first menstrual day and in a sample which had been obtained around day 21 in the preceding menstrual cycle (for details of the assay, see Batra *et al*, 1978). Vasopressin determinations were made on plasma extracted with bentonite (Skowsky *et al*, 1974) using a radioimmunoassay technique based on that of Chard and Forsling (1976). Iodination was accomplished by lactoperoxidase (Karonen *et al*, 1975).

Statistical evaluation of the results was carried out using the Mann-Whitney Test (Wilcoxon, 1945; White, 1952).

RESULTS

Data from women with plasma osmolality and sodium concentrations outside the normal range (260–310 mosmol/l and 136–146 mmol/l, respectively), from women who had not ovulated in the previous cycle as indicated by the plasma progesterone concentration at day 21, and from dysmenorrhoeic women who had no pain at the time of sampling were excluded. This left six women with dysmenorrhoea and five controls in the study. The vasopressin concentrations in the two groups on day 1 and day 5 to 7 of the cycle are shown in Table I. On day 1 the mean vasopressin concentration in plasma of women with dysmenorrhoea was approximately twice that in the controls, the difference being statistically significant ($p < 0{\cdot}05$). When all the available values from both sample I and II were used for statistical comparison, the difference became even more significant ($p < 0{\cdot}01$). It was also found that the vasopressin concentration in the controls on day 5 to 7 was significantly higher than during the day 1, both when the mean values for each day and all available values were compared ($p < 0{\cdot}01$ in both instances). In women with dysmenorrhoea the mean vasopressin level was also somewhat higher on day 5 to 7 than on day 1, although this difference did not attain statistical significance. Neither was there any significant difference between the plasma concentration in dysmenorrhoeic women and controls on day 5 to 7.

The plasma concentration of oestradiol in dysmenorrhoeic women during day 1 was 82 ± 12·1 (SEM) pg/ml, which was not significantly different from the values of the control group (66 ± 15·8 pg/ml). Neither was any significant difference observed in the plasma concentration of progesterone which was 0·40 ± 0·10 (SEM) ng/ml and 0·32 ± 0·05 ng/ml in the dysmenorrhoeic and control groups, respectively.

The plasma osmolality and plasma sodium

TABLE I

Vasopressin concentrations in plasma (μU/ml) in six women with dysmenorrhoea and five controls during day 1 and day 5 to 7 of the menstrual cycle

	Severity of symptoms	Cycle day 1			Cycle day 5 to 7		
		Sample I	Sample II	Mean	Sample III	Sample IV	Mean
	Light	0·32	0·67	0·50	0·83	1·26	1·04
	Medium	0·56	0·35	0·46	0·19	0·10	0·15
Women with dysmenorrhoea	Severe	0·35	0·30	0·33	0·47	0·44	0·46
	Severe	0·30	0·42	0·36	0·77	0·81	0·80
	Severe	0·26	0·28	0·27	0·22	0·18	0·20
	Severe	0·48	0·50	0·49	1·05	0·85	0·95
Mean		0·38	0·42	0·40	0·59	0·61	0·60
±SEM		±0·048	±0·046	±0·038	±0·143	±0·182	±0·154
		0·50	0·40	0·45	0·70	1·10	0·90
		0·17	0·20	0·19	1·00	0·80	0·90
Controls		0·11	0·12	0·12	0·60	0·57	0·59
		0·14	0·19	0·17	0·60	0·89	0·75
		0·06	0·15	0·11	0·26	—	0·26
Mean		0·20	0·21	0·21	0·63	0·84	0·68
±SEM		±0·078	±0·049	±0·063	±0·183	±0·215	±0·119

were not significantly different on day 1 and day 5 to 7, nor was there any difference between the control and dysmenorrhoeic women.

DISCUSSION

The present study demonstrated a significantly higher plasma concentration of arginine vasopressin in women with primary dysmenorrhoea than in healthy controls on the day 1 of the cycle. No differences in the plasma levels of progesterone and oestradiol were found between the two groups at this time. A second interesting finding was the significantly higher vasopressin concentration in the normal women on cycle day 5 to 7 compared with day 1. This difference was also seen in the controls excluded from the study, but not in the women with dysmenorrhoea, probably because the concentrations were already elevated on day 1.

It is not possible to decide from the present data whether vasopressin played a role in the genesis of dysmenorrhoea or if the increased vasopressin secretion, compared with that in controls, resulted from the menstrual pain. The fact that the subjects in the dysmenorrheic group had a higher vasopressin concentration on day 5 to 7 does not exclude the possibility that vasopressin played a role in the induction of pain on the first menstrual day through an action on the uterus. The uterine sensitivity to vasopressin varies in the menstrual cycle, and the effect is most pronounced at the onset of menstruation and least at the time of ovulation (Joelsson *et al*, 1966; Coutinho and Lopes, 1968; Bengtsson, 1970: Åkerlund and Andersson, 1976).

In recent studies on the cause of the myometrial hyperactivity in primary dysmenorrhoea, interest has almost exclusively been focused on the role of prostaglandins originating from the endometrium (see Halbert *et al*, 1976; Ylikorkala and Dawood, 1978). The successful use of drugs, inhibiting prostaglandin synthesis and action, in the treatment of dysmenorrhoea, together with the reduction of uterine hypercontractility which can be achieved by these drugs, has strengthened the belief in a role for prostaglandins in this condition. However, the concentrations of prostaglandins are elevated in some but not all patients with dysmenorrhoea, so that the prostaglandin theory cannot in all cases explain the myometrial hypercontractility associated with the condition (Halbert *et al*, 1976). Furthermore, other uterine stimulants might well act via final prostaglandin release

(Csapo and Csapo, 1974), and both uterine stimulants and other factors, such as the ovarian steroids, probably influence the synthesis of uterine prostaglandins (see Halbert *et al*, 1976).

Of the two posterior pituitary hormones, oxytocin seems to have a physiological role in stimulating myometrial activity during labour at full term (Chard, 1977). Vasopressin is a considerably stronger uterine stimulant than oxytocin in the nonpregnant condition, especially at the onset of menstruation (Joelsson *et al*, 1966; Coutinho and Lopes, 1968; Bengtsson, 1970). Warren and Hawker (1967) found that plasma oxytocin in 11 dysmenorrhoeic women, as determined by bioassay, was lower than that in matched control subjects. While this appeared to be a paradoxical finding, they concluded that when oxytocin levels are low, the uterine contractions, induced mainly by vasopressin, are dysrhythmic and painful. We are not aware of any studies on the plasma concentration of vasopressin in primary dysmenorrhoea, although there is a preliminary report suggesting a variation in vasopressin levels throughout the menstrual cycle (Skowsky and Swan, 1977 unpublished). The two principal findings of the present study, the difference in vasopressin concentration between dysmenorrheic women and controls on day 1 of the cycle, and a variation in vasopressin secretion, related to the cycle, should focus interest on the possible significance of this hormone both in reproductive physiology and in pathological conditions with menstrual disturbances.

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CHANGES IN COAGULATION FACTORS IN POSTMENOPAUSAL WOMEN ON ETHINYL OESTRADIOL

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Summary

Coagulation factors II, VII, X, VII + X complex and anti-thrombin III were measured in 10 postmenopausal women before, during and after a four-month course of 20 μg daily of ethinyl oestradiol, taken three weeks in four. Factors VII, X and VII + X complex rose significantly during treatment but only factor X levels were significantly elevated at the end of the four-month treatment period. There was no change in factor II or anti-thrombin III levels. These effects on coagulation factors during ethinyl oestradiol therapy are, in general, similar to those found during treatment with conjugated equine oestrogens and oestradiol valerate in a previous study from the same laboratory. It seems likely that equipotent doses of ethinyl oestradiol and 'natural' oestrogens have similar effects on these coagulation factors.

THERE has been speculation that 'synthetic' oestrogens such as ethinyl oestradiol and mestranol may carry a greater risk of thromboembolic disease than 'natural' oestrogens such as conjugated equine oestrogens, oestrone sulphate or oestradiol valerate (Editorial, 1974).

There are no epidemiological studies to support or refute this claim. Bonnar *et al* (1976) reported, however, that a mestranol-norethisterone preparation caused greater elevation of coagulation factors in menopausal women than either conjugated oestrogens or oestradiol valerate although the latter preparations did affect some coagulation factors. These differences could be due to a relatively higher oestrogenic potency of the synthetic preparation which contains varying doses of mestranol in the 28-day pack, reaching a maximum dose of 50 μg on two days. The differences in the effects of mestranol-norethisterone on coagulation factors compared to the other oestrogen preparations might therefore disappear if smaller doses of synthetic oestrogen were used.

To test this hypothesis, patients attending the same menopause clinic as the patients studied previously by Bonnar *et al* (1976) were given

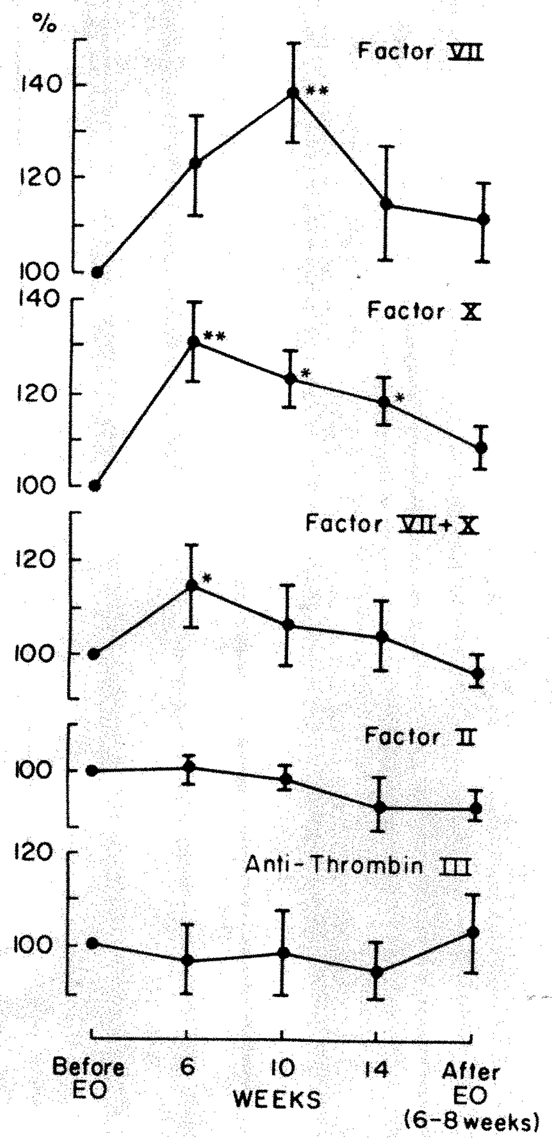

FIG. 1

Changes in coagulation factors VII, X, VII + X, II, and in anti-thrombin III before during and after ethinyl oestradiol (OE) 20 μg daily.
* $p < 0{\cdot}05$ ** $p < 0{\cdot}01$

the synthetic oestrogen, ethinyl oestradiol 20 μg daily, a dose apparently as effective in the relief of menopausal hot flushes as conjugated equine oestrogens, 1·25 mg daily, or oestradiol valerate, 2 mg daily—the oestrogen preparations used in the previous study. An identical protocol was followed in both studies and coagulation factors were measured by the same techniques (Biggs, 1972).

PATIENTS, METHODS AND RESULTS

Ten postmenopausal patients with hot flushes were given ethinyl oestradiol, 20 μg daily, three weeks out of four, for 16 weeks. Peripheral venous samples for coagulation factors II, VII, X, VII + X complex and the coagulation inhibitor anti-thrombin III were taken twice before treatment; in the sixth, tenth and fourteenth week of treatment, and six to eight weeks after therapy stopped.

The mean of the two pre-treatment values was called 100 per cent. All subsequent values for each patient were then expressed as a percentage of this pre-treatment value and the mean value for the 10 patients obtained. Statistical differences between means were tested by the Wilcoxon signed rank sum test for paired samples and the Mann-Whitney test for unpaired samples (Conover, 1971).

Figure 1 shows that there was a significant rise in mean values of coagulation factors VII, X and VII + X complex in the 10 patients on ethinyl oestradiol. Factors X and VII + X complex reached a peak after six weeks of therapy and then fell while the mean value of factor VII continued to rise to a maximum of 138 ± 11 per cent (mean ± SEM) by the tenth week of treatment. By the fourteenth week of treatment this level had fallen to 115 ± 12 per cent. Factor X was the only factor significantly raised at the end of 14 weeks treatment. There was no significant change in factor II and anti-thrombin III in the patients on the ethinyl oestradiol.

DISCUSSION

It is clear that 20 μg daily of ethinyl oestradiol in postmenopausal women significantly elevated coagulation factors VII, X and VII + X complex within 6 to 10 weeks of starting therapy, but factors VII and VII + X were no longer elevated after 14 weeks' treatment. The changes in factor X on ethinyl oestradiol are similar to those previously reported (Bonnar *et al*, 1976) during mestranol-norethisterone therapy, although the rise was greater after the mestranol preparation, reaching 162 per cent of the pre-treatment level after eight weeks' treatment.

Compared to coagulation factor levels in patients on conjugated oestrogens or oestradiol valerate (Bonnar *et al*, 1976), mean levels in patients on ethinyl oestradiol were no different for factors II, VII, VII + X complex and antithrombin III. Factor X levels 6 and 10 weeks after ethinyl oestradiol were significantly higher ($p < 0{\cdot}01$ and $< 0{\cdot}05$ respectively) than in patients reported by Bonnar *et al* (1976) treated by conjugated equine oestrogens 1·25 mg daily, but were no different at any time to levels on oestradiol valerate 2 mg daily.

The dose of synthetic oestrogen in this study and of 'natural' oestrogens in the previous report (Bonnar *et al*, 1976) is greater than the dose required to relieve hot flushes in most patients and it is possible that the changes in coagulation factors would be less if smaller doses of oestrogens were used.

Epidemiological evidence is lacking, but it may be that the dose of ethinyl oestradiol required to control menopausal symptoms is no more thrombogenic than the equipotent dose of 'natural' oestrogens, although, of course, many other aspects of clotting mechanisms such as effects on lipoproteins, platelets and the fibrinolytic system should be taken into account. In view of these preliminary results and because of the relative inexpensiveness of ethinyl oestradiol compared to other oestrogen preparations used to treat menopausal symptoms it is important to continue to evaluate the use and safety of ethinyl oestradiol in the treatment of menopausal patients.

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A REPORT OF THE MEETING OF THE BLAIR BELL RESEARCH SOCIETY AT THE ROYAL COLLEGE OF OBSTETRICIANS AND GYNAECOLOGISTS ON 11th DECEMBER, 1978.

Six papers were presented and discussed.

The Outcome of Spontaneous Labour in Different Racial Groups was presented by Dr Margaret Thom on behalf of herself, Dr Chan and Mr J. Studd, of King's College Hospital. The progress and outcome of spontaneous labour was examined in 1643 patients, relating these to three broadly divided ethnic groups—Caucasion, Negro and Asian. Cervimetric progress was assessed with a Studd partogram: graphs from 42 per cent of the primigravidae and 26 per cent of the multigravidae fell on the delayed side of the mean line. All racial groups gave birth to babies with lower one and five minute Apgar scores and heavier birth weights when their graphs were on the delayed side of the line. Caesarean section rates were also increased, being 7·6 per cent and 18·2 per cent for white and Negro primigravidae respectively. It was noted that Negro multigravidae had a higher Caesarean section rate irrespective of the relationship of their partogram to the norm because of complications such as hypertension, fetal distress and placental abruption. In discussion, the cross correlations of age and social class were considered as well as the factors of race. The grouping of people from widely different geographical areas in the one race was queried and concern was expressed about studies where one group was very large and the compared groups were small in numbers.

Fetal Breathing Movements in Normal Antenatal Patients in Response to 5 per cent Carbon dioxide Inhalation was submitted by Dr J. Knox Ritchie and Dr K. Lackarney of Belfast, the former author presenting the paper. In the last 10 weeks of pregnancy, fetal breathing movements and generalized fetal body movements were recorded for 15 minutes and then a gas mixture of 5 per cent CO_2 and 95 per cent air was given by face mask; a further 15 minutes of fetal trace was then made. The most noticeable change was that fetal breathing movements increased almost three-fold after CO_2 administration. The other changes in the fetus, such as heart rate, were not so noticeable and the maternal blood gas analysis showed that with a small rise of CO_2 tension, there was also an increase in O_2 tension because of the overbreathing recorded. The discussion of this paper was taken with the following linked paper.

Dr Peter Boylan and Dr Peter Lewis of the Institute of Obstetrics and Gynaecology presented a study of 19 patients on *Human Fetal Breathing Movements in Labour*. This was studied both antenatally and during labour in patients with a normal perinatal outcome. Whilst breathing movements were seen in all fetuses in the antenatal period, only one-third showed such movements in labour. A similar reduction of all fetal movements was seen. Since many of those in labour were receiving intravenous dextrose, the relation between plasma glucose levels and fetal breathing was considered and shown to be related but no such relation was shown with other fetal movements. The lack of breathing movements in the newborn was also considered in relation to plasma levels in the mother. In discussion the usefulness of fetal breathing movements as a measure of fetal well being antenatally and in labour was considered. It was felt that standardisation presented such problems that fetal breathing movements might not be useful in practice. The stresses of raised blood sugar and CO_2 breathing were considered but more work on standardisation was required before this could be a useful practical test.

Dr M. Singh presented the work he had done with Dr F. Anthony, Dr P. Woods in Southampton on *Pregnancy-specific* β_1*-Glycoprotein* (*SP_1*) *Levels in Relation to Asymptomatic Glucose Intolerance in Pregnancy*. It had been suggested that SP_1 might act as a regulator of carbohydrate metabolism. The group set out to determine if there were any differences in SP_1 levels in patients who had normal pregnancy, those labelled as having chemical gestational diabetes and those with potential diabetes. A fasting and a two-hour plasma glucose sample was assayed after a load of 50 g of oral glucose to determine if a greater number of patients who had an abnormal glucose tolerance had SP_1 levels above the mean. This was shown to be so but was not due to the extra weight of the baby or that of the placenta. There was discussion about the cut-off points at a mean level and it was wondered if a distribution curve analysis would possibly have been more useful as a discriminant.

The Treatment of Amenorrhoea Resulting in Normal Fertility was presented by Dr M. G. R. Hull on behalf of himself, Dr T. Savage and Dr H. Jacobs. This work came from the Bristol Maternity hospital. A straightforward investigation and treatment of amenorrhoea and oligomenorrhoea was described. If the condition was not due to primary ovarian failure, treatment was applied as appropriate with clomiphine (52 per cent of the population) bromocriptine (25 per cent) or gonodatrophins (20 per cent). In all, 59 patients were treated and all ovulated. It was reported that 93 per cent had conceived at the time of the investigation but it was too soon to make any comments about term pregnancies for 68 per cent of the women were still currently pregnant. Within the practice of this unit, 79 per cent could expect to have conceived by the sixth cycle, and 94 per cent by the 12th cycle of treatment. The multiple pregnancy rate was 13 per cent and the abortion rate 22 per cent. These figures equalled the best reported for normal women.

Moderate Hyperprolactinaemia in Infertile Women was presented by Dr Derek Lo. Mr W. Cooper, Miss M. C. Browning and Mr John Mills from The Medical School, Dundee were the collaborators in this study of eight infertile regularly menstruating women with plasma prolactin levels above 500 mU/l and below 1000 mU/l. In these patients, oestradiol values in the second half of the cycle were significantly lower than the control group of women with proven fertility and normal levels of prolactin. There was no difference in gonadotrophin levels. Bromocriptine therapy resulted in a slight increase in the levels of oestradiol and progestogen secretion suggesting that a luteal phase deficiency was associated with moderate hyperprolactinaemia.

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BOOK REVIEWS

British Births 1970—Vol. 2. Edited by G. CHAMBERLAIN. Pp xiv+292, with numerous figures and tables. Wm Heinemann, London, 1978. Price: £12.00.

THE first volume of British Births published in 1975 recorded the condition of 17 196 babies born after 24 weeks gestation in a week in April, 1970. Volume 2 continues the story and describes the obstetric care given to the mothers. Despite being primarily a volume of statistics, the text, written by two practising obstetricians, reads easily and is spiced at times by the introduction of the authors' own opinions. Inevitably, comparison is made with the 1958 National Birthday Trust survey and emphasis is placed where fields of ignorance still exist as in the aetiology of antepartum haemorrhage and hypertension. Where antenatal care and management of labour relate directly to fetal outcome, attention is paid to the quality of survival and not just life. The last chapter has been prepared by members of the Committee of the National Birthday Trust and summarizes impressions. The main conclusions drawn are that, in relation to the 1958 survey, there is an increasing social class difference in both care and outcome for pregnant women. There has, up until 1970, been no decline in the incidence of small babies, in fact the opposite, with a relative increase in babies born to poor low income households as the number of poor homes increases and the number of babies born in Social Classes I and II households decreases. Recommendations include the maximum application of existing knowledge to high risk groups and the identification of the problem of persuading those in this group to accept help by making it both easier to obtain and perhaps financially rewarding. More resources for the maternity services are urged and more support for those families with low living standards who are most at risk. On the research side, more attention to antepartum haemorrhage, hypertension and pre-term babies is urged. The editors draw attention to the fact that authorship changed when the bulk of material had already been analysed and this is no doubt one reason why reading the book in 1978 makes some of the findings seem somewhat dated. This is particularly true in regard to the chapters on labour where the introduction of monitoring techniques and epidural analgesia, and increased awareness of the labouring woman's own wishes have led to marked changes in the last eight years which would no doubt produce very different results and conclusions if the study were repeated now—whether the changes would all be for the better, only another nationally based study will show. The book comes at a time when many obstetricians and paediatricians are finding pressure on their existing resources and provides an excellent review of some of the problems facing our profession.

Joan Andrews

Contraceptive Technology, 1978-1979. 9th Revised Edition. Edited by R. HATCHER, G. K. STEWART, F. STEWART, F. GUEST, P. STRATTON and A. WRIGHT. Pp v+192, with numerous figures and tables. Irvington Publishers, Inc, New York, 1978. Price: $3.95.

THIS book comes from the Emory University Family Planning Program, Atlanta, Georgia. It is written for clinicians, the subject matter is comprehensive, covers all methods of birth control, is well classified and easy to read. Nevertheless, viewed as a whole, the book is a disappointment chiefly because its quality is so uneven. Sometimes the literature is competently reviewed, but too often statements seem to be based on clinical impressions without this being clearly stated. Some sections, such as that on intrauterine contraception, are good. But Chapter 4 on oral contraceptives is disturbing, even after allowing for the differences between Britain and the USA. Most family planning doctors would query a number of the statements. For example, pills containing more than 50 μg of oestrogen would never be recommended. On page 47 a strange regime, "Start your first pack on the first Sunday after your period begins" presumably accounts for the unusual advice to use a supplementary method during the whole first month on the pill. What is the evidence for the pill causing "autophonia, chronic dilation of the eustachian tubes rather than cyclic opening and closing" (page 51)? And while definitely agreeing that "education is the key to the initial visit" British doctors will be staggered by the statement: "If the initial visit takes more than two hours, a patient may be reluctant to return" (referring to the consultation time). The authors' confidence in the effectiveness of spermicides used alone (as foam, or the Encare Oval) also seems misplaced. Considering the 1979 in its title, the book is not very up-to-date: clip and ring methods of sterilization are dismissed in one sentence as insufficiently tested. The short sections of suggested patient instructions about most of the methods are interesting. They are much more detailed and potentially worrying to the user than those normally given in Britain, although apparently quite acceptable and becoming medico-legally unavoidable in the USA. Misprints are rather frequent and not always unimportant: the *intra*-uterine position of the Verres needle is apparently to be ascertained before 3 litres of CO_2 or N_2O are infused prior to laparoscopic sterilization.

J. Guillebaud

Prenatal Diagnosis of Genetic Disease. By D. C. Siggers. Pp x+69, with numerous figures and tables. Blackwell Scientific Publications, Oxford, 1978. Price: £3.25.

This book is an admirable brief introduction to prenatal diagnosis of genetic and congenital disorders. Its chapters deal clearly with the main areas of prenatal diagnosis: chromosome disorders, neural tube defects, inherited biochemical disorders, genetic counselling, X-linked disorders, amniocentesis and application of techniques other than amniocentesis for prenatal diagnosis. Two appendices list centres in the UK providing genetic counselling and laboratories with facilities for prenatal diagnosis. References are listed at the end of the book which concludes with an index. The treatment of this comprehensive range of topics is inevitably brief and didactic. Nevertheless it omits no essential points and is remarkably up to date with references up to 1977, but in a subject developing so rapidly the book will soon become out of date and the publishers will have to accept the necessity for fairly frequent revision.

M. Crawford

Amniotic Fluid. By D. V. I. Fairweather and T. K. A. B. Eskes. Pp xii+468, with numerous figures and tables. Excerpta Medica, New York, 1978. Price: $78.25.

The remarkable growth of interest in, and information about, the amniotic fluid has made it a diagnostic oracle for obstetricians which rivals the place held by urine for the medieval physicians. The second edition of this multi-author compendium, appearing only four years after the first, has been considerably enlarged and revised to take account of the rapid accumulation of knowledge. For example, a criticism of the first edition was that phospholipids in amniotic fluid should have been afforded a chapter to themselves; in this edition two chapters are given to them: an excellent review of laboratory techniques by Mr T. I. Wagstaff and one on clinical application by Professor C. R. Whitfield. Thirty authors from the UK, Europe and USA have covered the origin, volume and pressure of amniotic fluid, its biochemistry and cytology, its reflection of fetal maturity, disease and abnormality, even amniotic fluid embolism. Each chapter is a well documented review and as up-to-date as is possible for a textbook in a fast moving subject. Inevitably, this means that important developments since the chapters were written are not included and it is a pity that Professor Fairweather's own chapter on technique and safety of amniocentesis had to be written before the recent report of a study by the Medical Research Council on hazards was published. All in all this is a most valuable collection and every obstetrician would benefit from having it at his elbow.

F. E. Hytten

Clinics in Endocrinology and Metabolism, Reproductive Endocrinology. Edited by G. T. Ross and M. B. Lipsett. Pp v+200, illustrated. W. B. Saunders Company Ltd, East Sussex, 1978. Price: £8.25.

If this book was poetry it would be called a slim volume. As it is, it is thin, both in size and matter. W. B. Saunders company have been bringing out their 'Clinics in Endocrinology and Metabolism' at a brisk rate and the latest one on Reproductive Endocrinology has a somewhat tired air. Griff Ross and Mortimer Lipsett who edited this book are major figures in the world of endocrinology and have sufficient influence to have recruited a few big names. Unfortunately, although you can lean on someone sufficiently to make him do an article, there is no way of ensuring that the writing will be fresh and good. For the most part these are rather uninspired rewrites which give the impression of having been done as a chore. If they did not interest the authors, it is unlikely that they will interest the reader. It might be supposed that reproductive endocrinology lies close to the province of obstetrics and gynaecology and that a book with clinics in the title would be relevant to clinical practice: it is not and only two of the 19 contributors can be recognized as gynaecologists. One of these, Robert Shaw of Birmingham, is the only British author and has contributed a straightforward account of the neuroendocrinology of the menstrual cycle. It is an involved subject and he makes it understandable without getting lost in the complexities of hypothalamic feedback control. If your library takes the 'Clinics' series, read it. The rest of the book is unnecessarily hard going and beside the point for the clinician.

A. Klopper

Cardiotokographie, Lehrbuch und Altas. Edited by Wolfgang M. Fischer. Pp xvii + 508, with 216 figs (565 separate illustrations). George Thieme Verlag, 7, Stuttgart 1, P.O. Box 732, Herdweg 63, Germany, 1976. Price: Geb. DM. 98,-.

Tests to assess fetal well-being underpin obstetric management in pregnancy and parturition. Only cardiotocography is applicable in both circumstances; further, it has the unrivalled merit of giving up-to-the-minute information about the fetus but this demands accurate clinical interpretation. However, before the clinician can usefully employ any fetal monitoring he must familiarize himself with and be able to weigh-up all available techniques. In order to utilise cardiotocography he must be conversant with its technicalities and understand the range of uterine motility as well as fetal cardiovascular physiology and its aberrations when under the stress of natural or contrived uterine activity. This book affords him all of this and also aids in the interpretation of cardiotocographic tracings with a chapter of 150 pages devoted to practical work-a-day examples. There is a final thought-provoking chapter discussing the concept of a cardiotocographic on-line monitoring system to try and minimize the effects of observer misinterpretation. Thus the reader gets a balanced view of current fetal monitoring methodology as well as a thorough insight into cardiotocography, all against a background of a comprehensive review of the relevant world literature. An English translation really must be set in train.

E. G. Jonas

Clinics in Obstetrics and Gynaecology. Gynaecological Urology. Edited by S. L. Stanton. Pp vii + 248 with illustrations. W. B. Saunders Company Ltd, Eastbourne, 1978. Price: £8.25.

All gynaecologists will have referred to them patients whose primary problem is one of malfunction of the lower urinary tract. It is within this context that we must judge the latest in the series of Clinics in Obstetrics and Gynaecology. As with many review books of this type, the gynaecologist who has made a study of this subject will find little new material but for the majority it offers a valuable insight into gynaecological urology in one small volume. One problem with volumes containing contributions from multiple authors is that there tends to be considerable repetition. Here the authors have kept fairly well to their subjects although the method of videocystourethrography with pressure studies is described more than once. The plea by the editor for standardization of terminology and units of measurement is important. I was therefore surprised to note that in the first chapter some cystometric traces were calibrated in millimetres of mercury and others in centimeters of water, which can be confusing. To be fair, however, this was the only such anomaly within the book. The difference in approach between Europe and the USA is highlighted in Chapter 2; in Europe most workers use fluid cystometry whereas in the United States, with the greater emphasis on office procedures, gas cystometry is more popular; a more detailed comparison of the two would have been welcome and informative. Helpful hints on which patients to investigate are provided and the chapter on voiding problems provides a useful classification at the same time as dispelling a number of myths. The chapters on congenital abnormalities, urinary tract infection, surgical injuries and surgery for incontinence are clear and comprehensive, offering useful reviews of the subjects. In summary, this volume offers practical advice at the same time as pointing out the deficiencies in our knowledge. The small amount of time required to read it makes it worthwhile for most practising gynaecologists as well as helping students to place the problems in perspective.

J. L. Osborne

Perinatology Case Studies. By L. Iffy and A. Langer. Pp 576, illustrated. Henry Kimpton Publishers, London, 1978. Price: £13.50.

Good textbooks outline principles, and suggest the basis for diagnosis and treatment. This book outlines the diagnosis and treatment of individual cases, with succinct descriptions of the principles derived from recorded experience which underlie their management. There are 85 of these cases, which cover all of the commonly encountered problems in obstetrics and some of the usual neonatal complications. Each condition is fully reviewed, and in some cases there are flow diagrams of the action to be taken. References for further reading are plentiful, and include many from the British literature as well as from the North American. Management is certainly acceptable on this side of the Atlantic. The chapters being shorter than in a formal textbook, this book can be read with profit even if only little time is available.

I. R. McFadyen

Clinics in Obstetrics and Gynaecology. Gynaecological Surgery. Edited by D. H. Lees and A. Singer. Pp v + 246, illustrated. W. B. Saunders Company Ltd, Sussex, 1978. Price: £8.25.

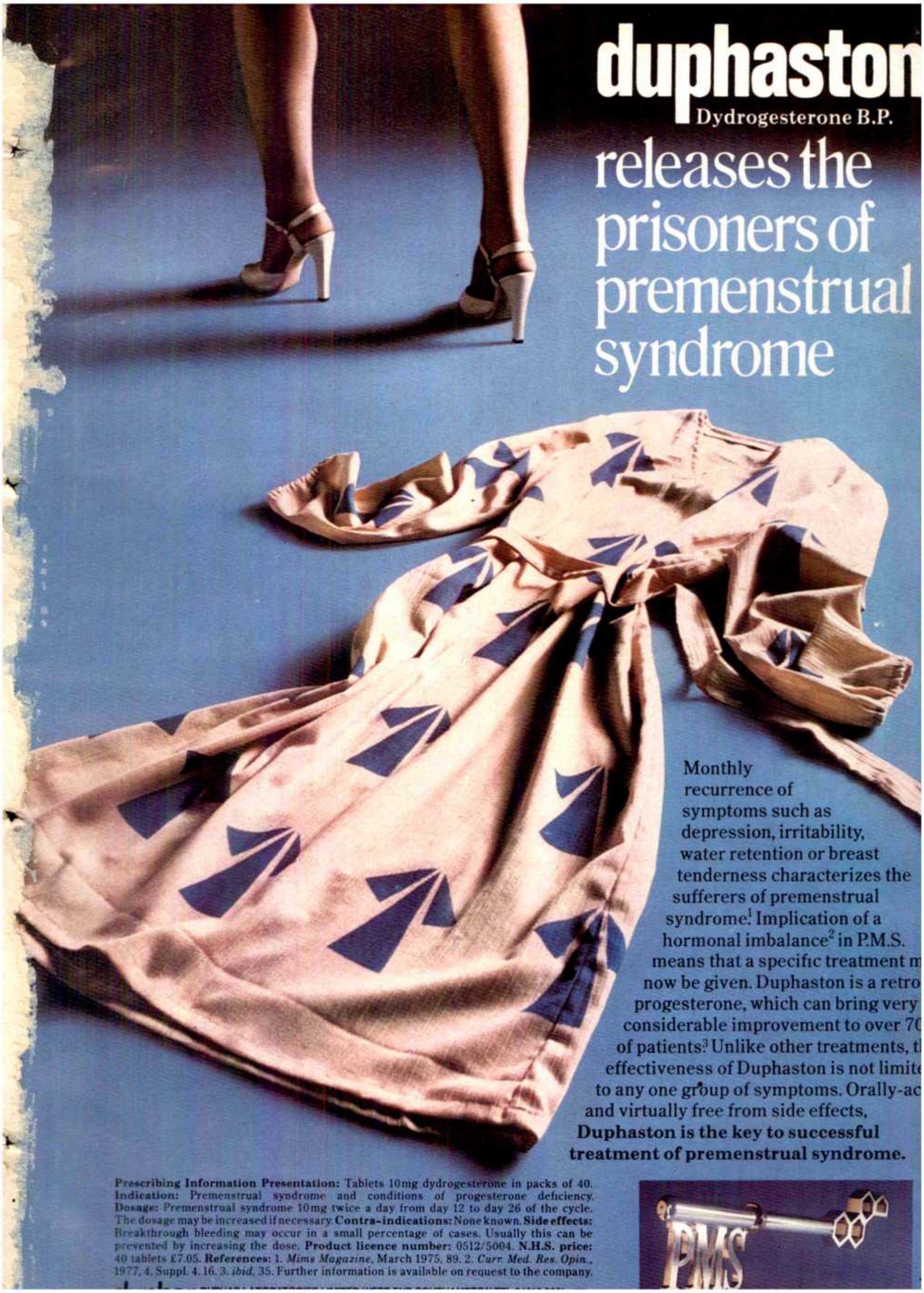
The December 1978 volume of 'Clinics' is devoted to gynaecological surgery and the subjects discussed cover almost the whole range of gynaecology. In the foreword, the guest editors suggest it should be possible to say which of the traditional surgical methods are worth retaining and which should be discarded, but there is nothing obviously relegated to the discard pile. A wide selection of authors offer an authoritative, up-to-date, comprehensive review and even if some protest a little too loudly that theirs is the best or only way, others are properly sceptical of some published results and opinions. The result is that it all makes for good reading with plenty of scope for personal interpretation and discussion and argument with one's colleagues. All the chapters are illuminating, stimulating and easy to read, and it seems churlish to criticize anything in such a uniformly good collection. However, extensive use of clinical photographs, although expensive to reproduce clearly even in black and white, seems to add very little to and may indeed divert one from a well written description. The line drawings in some of the chapters often make the point much better. All the contributors should be congratulated on this volume and I recommend it without reservation as an undeniably good buy.

D. W. T. Roberts

Errata: Vol. **86,** p. 251

A Report of the Meeting of The Blair Bell Research Society at the Royal College of Obstetricians and Gynaecologists on 9th October, 1978. *Rapid Whole Blood Lactate Measurements in Fetus and Mother during Labour*, presented by N. Smith *et al.*

The whole blood lactate levels were actually measured in mmol/l, NOT in nmol/l as printed.



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c antidepressants and deep inhalation anaesthetics which of themselves may be arrythmogenic. Recent reports describe pulmonary oedema in patients treated concomitantly
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